Understanding the Phenotype of Aggressiveness

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PREFACE

"Pigs are very beautiful animals. Those who think otherwise are those who do not look at anything with their own eyes, but only with other people's eyeglasses."

(Chesterton, 1908)

Dedication

To Michael, Diane and Andrea Clark, who made this possible through their constant encouragement and support. Also, the other members of my family, Margaret Clark, Richard Hibbitt, May Percy and David Irvine.

This thesis is dedicated to the memory of some truly inspirational people, Gladys Barkham, Muriel Hibbitt and Bryan Marwood.

Declaration

I hereby declare that this thesis is of my own composition and that all assistance has been duly acknowledged. The results presented herein have not previously been admitted for any other degree or qualification.

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"*Blessed are the meek: for they shall inherit the earth*"

(Beatitudes, Matthew 5:5)

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Abstract

Mixing together unfamiliar pigs is a common husbandry practice in commercial indoor piggeries that causes a period of intense fighting and aggression between pigs, resulting in elevated stress responses and injuries to those involved. Frequency, duration and intensity of aggressive behaviour have been found to differ between individuals. There is some evidence that these differences are due to trait-aggressiveness, as consistency of responses to similar challenges has been demonstrated. This trait-aggressiveness may be linked to other behavioural (social and non-social) and physiological traits to form part of personality. To understand aggression in the context of behavioural trait the prevalence of aggression was studied throughout the lifetime of a cohort of pigs, examining consistency within and between situations at numerous points. More specifically, the aims of this thesis were to investigate: whether the relative magnitude of aggression displayed in response to a repeated social challenge test (Resident Intruder Test) was consistent over time; if this aggressiveness changed with age and experience; whether RIT aggressiveness was comparable to, or predictive of aggressiveness during social mixing; what strategies were employed by pigs of differing aggressiveness during mixing; and finally, if aggressiveness was linked to the response of individual pigs to a challenging environment, unconnected to social confrontation (maternal behaviour).

Detailed behavioural analysis was performed on the progeny of 19 gilts. Consistency of aggressiveness was examined using repeated Resident Intruder Tests at various key stages during the lifetime of male and female growing pigs, and a subset of female breeding pigs. Behaviour during mixing at weaning and as gilts was examined in detail; information was collected about the way in which these animals behaved in a social context, including non-aggressive social behaviour, and how this related to injuries received (lesions). As social dominance status is often cited as a parameter related to aggressiveness, this was recorded at various stages using a group feeding competition test and compared to behavioural measures from mixing and the RIT. To gain a better understanding of the interaction between aggressiveness and other traits, the various measures of aggressiveness were compared with cortisol (as a physiological indicator of the relative stress responses of individuals) and the behaviour of a subset of female pigs in the period immediately preceding and following farrowing. Maternal behaviour was chosen for comparison to aggressiveness as it is also potentially a trait-behaviour, specific to individuals, that is not obviously linked to social

aggressiveness; but also because it is both important commercially and for the welfare of the gilts and piglets.

Pigs were consistent in their responses to the RIT, but there were differences between sexes. RIT aggressiveness was consistent over a long period of time in female pigs, even with a gap of 90 days between tests and the onset of puberty. Male pigs showed an unexpectedly high level of mounting behaviour from a young age, which increased with maturity. Experience of the RIT improved consistency of responses, and age at first testing affected both the speed of attacking and occurrence of attacks: those pigs experiencing the test when younger were more likely to and quicker to attack. Although aggression in the RIT was consistent, it was not predictive of subsequent aggressiveness at mixing.

As with the RIT, there were clear sex-differences observed during mixing at weaning, with males being more aggressive, more successful in fights, more likely to mount and less likely to play than females. Pigs employed different strategies during mixing, the extremes of which were categorised by high-play-low-aggressiveness and vice versa. As expected, aggressive individuals were involved in more fights and won more fights, but suffered more skin lesions than non-aggressive individuals. Pigs that engaged in high-playing were generally the least successful in fights, but suffered fewer lesions and had equal ultimate dominance rank to aggressive pigs. The behavioural structure of mixing changed between weaning and puberty, with differences in the occurrence and duration of aggressive and non-aggressive behaviours. Fighting ceased sooner during the gilt mix, but aggression was more frequent and more severe. Comparisons with maternal behaviour found gilts that reacted aggressively to their piglets were more aggressive and successful in the mix and more 'reactive' during farrowing. There were other links between farrowing and mixing behaviour, such as more frequent posture changes but less frequent nesting with greater mix-aggressiveness; indicating that aggressiveness and maternal behaviour traits are linked through personality.

The RIT was not a reliable predictor of aggressiveness, particularly in male pigs. Aggressiveness did not always equate to status as some individuals managed to attain rank without severe fighting or receiving excessive numbers of lesions. It may be that aggressive pigs are more reactive in a number of situations, reactivity to piglets during farrowing being one of these situations. The results indicate that aggression should not be viewed in isolation, but with other traits that make up personality. Rather than being a fixed entity, aggressiveness develops with age, sex, experience and environmental influences.

CHAPTER 1

GENERAL INTRODUCTION: BACKGROUND, OBJECTIVES AND OVERVIEW OF EXPERIMENTAL CHAPTERS

1.1 Aggression

Aggression is a fundamental part of the social behaviour of many species. It forms the basis of social group organisation and stability (Drummond, 2006), although it can also lead to negative consequences for both the aggressor and recipient, such as stress (Abbott et al., 2003), injury/illness (Hessing et al., 1994a; O'Connell et al., 2004b) and restricted access to resources (food, bedding and so on) (Schonfelder, 2005). Aggression is a behaviour that animals can employ to drive away others that may be a threat to them or their offspring and therefore, in wild conditions aggression can be an extremely effective strategy. Aggression between farmed pigs is however, a problem that has both welfare and economic consequences, even though it may perform a functional purpose in a different setting. Although aggressive behaviour is common within the confined setting of indoor piggeries, it is not performed to the same degree by all pigs (Meese & Ewbank, 1973; Erhard et al., 1997). This thesis seeks to understand whether the relative magnitude of aggression displayed in response to social challenges is a constant characteristic of individuals, which can be called aggressiveness, or if aggression is simply a transient or momentary reaction dependent on and altered by, specific social and physical environmental conditions.

1.1.1 Aggression - the welfare concerns in farmed pigs

The main source of excessive aggression between indoor-reared pigs occurs when unfamiliar pigs are mixed together to form new social groups (Arey, 1999). Pigs can be mixed at various points throughout their lives for management purposes. For example, pigs might be mixed to create uniform sized groups in order to maximise space usage, or to form same-weight groups that will attain target slaughter weight simultaneously. Piglets are commonly mixed at weaning, which in the UK is typically at around one month old (EU commission directive EU/2001/93/EC states the minimum age for weaning is 28 days, effective from 2003). This involves separating the sows and piglets with the latter then often mixed into new groups containing numerous litters of piglets.

The process of mixing unfamiliar pigs is generally considered to be detrimental to their welfare as it results in considerable aggression and as a consequence of which, many pigs receive wounds to their bodies (McGlone, 1985), referred to as skin lesions (Turner et al., 2006). Mixed pigs show responses indicative of high levels of stress (Otten et al., 1999; Merlot et al., 2004;), which may subsequently have a negative impact on reproduction

(Madej et al., 2005), immunity (de Groot et al., 2001) and meat quality (Terlouw et al., 2005).

In contrast to the aggression seen in intensively reared pigs, wild or free-living domestic pigs show very little aggression, even when piglets are first introduced to the social group (Newberry & Wood-Gush, 1986; Petersen et al., 1989). There are however substantial differences in the social and physical environments (for descriptions of feral/wild pigs see Graves, 1984; Gonyou, 2001). Wild sows live in family groups, with related females and their young of varying ages. The sow leaves this group shortly before giving birth (farrowing). She will locate a suitable site where she can build a nest in which to give birth and spend the first 10-14 days post-parturition. Although the sow may leave the litter and return to the social group for short periods, the piglets remain in the nest. After 10-14 days the nest is abandoned and both the sow and her litter join her original social group. The piglets remain close to the sow for some time after this, although they are exposed to and have numerous opportunities to mix with unfamiliar piglets. During this process of social integration commonly observed behaviours include nosing and play (Petersen et al., 1989). Fighting is rare; there may be some mild aggressive behaviour, such as butting, but this is often short lived and may be performed as a part of playful interactions (Newberry & Wood-Gush, 1986).

Standard commercial indoor systems significantly limit the opportunities for pigs to display normal behaviour patterns. This includes the use of farrowing crates, which impede the natural behaviour of the sow during parturition (Lawrence et al., 1997; Jarvis et al., 2004). As the sow is confined to the extent that movements beyond standing up and lying down are prevented, she is unable to temporarily leave or move away from the litter. In addition, the gradual introduction of piglets into social groups is prevented. Piglets are also weaned earlier than in natural conditions and when they first encounter unfamiliar pigs it is without the protection of the sow, or space to flee. Group structure is artificial, as there is often little variation between pigs in age/weight. After their litters have been weaned, the sows are usually mixed with unfamiliar sows of differing parities. In some cases social groups are entirely fluid, with the constant removal and addition of individuals (O'Connell et al., 2004a). In addition to the social environment, there is also considerable behavioural restriction resulting from the physical environment, with a lack of space, environmental complexity (e.g. foraging material) and sometimes food (in breeding animals), which have

all been linked to increased aggression (O'Connell & Beattie, 1999; Turner et al., 2000; Cox & Cooper, 2001).

1.1.2 Aggression and trait-aggressiveness

It is important to make the distinction between aggression and aggressiveness. Aggression is a complex behaviour, with no single functional or causal explanation; although an aggressive act may be immediately recognisable, producing an unambiguous definition can be problematic, as behaviour recognisable as aggression can vary widely in severity from assertiveness, to overt attacks and fighting. Aggression can be defined in terms of the motivation driving the behaviour (e.g. defensive aggression) (Rushen & Pajor, 1987), the functional outcome (e.g. territorial aggression) (Switzer et al., 2001) or the amount of forethought (e.g. premeditated aggression) (Ramirez, 2006). These categories are not mutually exclusive.

"*Aggression is the act, aggressiveness is the trait and anger is the emotion*" (Cook, 1993).

As illustrated above, performing an aggressive act is not the same as being an aggressive individual. A 'state' behaviour is the current response by an animal to any situation and the performance of an act of aggression (state aggression) can occur irrespective of whether an individual has a propensity to be aggressive or not (trait aggression). If the same state occurs in similar situations then it may be referred to as a trait of that animal. To demonstrate the existence of trait aggression there must be observable/definable differences between individuals, consistency within individuals, repeatability within the same testing situation and some predictability across different situations that would be expected to be motivationally similar (e.g. different types of social confrontation).

The term 'individual differences' has been used in a dismissive sense to define differences between experimental animals that are not explained by treatments (Erhard & Schouten, 2001), without specific discussion of their importance. However, differences in strategies within a population are well acknowledged. For example, for a number of years the existence of 'coping styles' has been reported in the literature. These are a collection of trait behaviours that have been used, particularly in rodent, and more recently (and more controversially) in pig studies, to describe an individual's reaction to a challenging social or non-social situation (e.g. Benus et al., 1990; Benus et al. 1991; Hessing et al. 1993; Wechsler

1995). Rodents have been classified as short (SAL) or long attack latency (LAL) (van Oortmerssen & Bakker, 1981), translated as individuals of high and low aggressiveness respectively. This aggressiveness corresponds with general patterns of stress-reactivity referred to as active/proactive or passive/reactive, with diverging physiological responses to challenges (Schuurman, 1980; Bohus et al, 1987; Fokkema et al, 1988; Sgoifo et al., 1996) and associated pathologies (Fokkema et al., 1995; Bolhuis et al., 2003; Campbell et al., 2003). In addition to being more aggressive, active copers (SAL) are generally characterised by high levels of locomotor activity, a high propensity to develop routine-like behaviour (linked to low responsiveness to environmental change) and high testosterone and sympathetic activity. Passive coping (LAL) is characterised by freezing responses, low locomotor activity, high flexibility of behaviour and responsiveness to external cues, and high HPA (Hypothalamic-Pituitary-Adrenal axis) and parasympathetic reactivity. If a group of such traits enable predictions about future behaviour and the occurrence of other traits, then these can be referred to as a 'type' (Cook, 1995). Evidence suggests that the distinctions in rodents between proactive and reactive coping styles represent distinct differences between 'types' (Koolhaas et al, 1999), with perhaps the most common method used to categorise these type extremes, being measures of individual differences in aggressiveness.

An alternative approach to categorising individuals along a single dimension of traits or types has been to examine behaviour at its highest level of organisation, at the level of personality. From a holistic perspective, an individual is a complex interaction of personality traits/types. The classic method of assessing personality in human psychology uses the Five Factor Model of Personality, the dimensions of which are: extraversion; agreeableness; conscientiousness; neuroticism and openness. This "integrated personality model" uses combined assessments of each axis to make predictions about behaviour, health and mental health of individuals (Korotkov & Hannah, 2004). The emphasis is therefore not in subdividing parts personality, but in understanding how the various traits combine and interact. This model has been reviewed across animal species (Gosling & John, 1999). It has been suggested that the same factors can be extracted, although as all five factors were only found in certain primate species it would seem that the complexity of social organisation is an important factor.

Although this thesis concentrates of trait-aggressiveness in pigs, aggression as a part of personality has been studied in many other species. Personality/temperament has been described in dogs for example (e.g. Svartberg & Forkman, 2002; Strandberg et al., 2005),

often to enable selection based on specific traits (including aggressiveness), for breeding Wilsson & Sundgren, 1997), as pets (Weiss & Greenberg, 1997) and as service animals, such as guide-dogs (Serpell & Hsu, 2001). Aggressiveness in other domesticated species has been well studied, including cats (Feaver at al., 1986), chickens (Rushen, 1984), horses (Arnold & Grassia, 1982) and cattle (Plusquellec et al., 2001; Schrader, 2002; Mülleder et al., 2003). Individual differences in aggression have also been reported in fish (Francis, 1990), squid (Sinn & Moltschaniwsyj, 2005) and spiders (Sih et al., 2004). Because of the links often made between human and primate behaviour it is perhaps not surprising that aggression has been studied in species including chimpanzees (Buirski et al., 1978) and rhesus macaques (Higley et al., 1996; Capitano, 1999). As mentioned previously, mice have been studied exhaustively with respect to individual differences in aggressiveness and how this relates to other traits that form a reactivity-type in coping with challenges. But it has also been proposed that these SAL and LAL lines can be used as an animal model for the purposes of finding genetic and neurobiological markers of antisocial behaviour in humans (Sluyter at al., 2003).

Although a well-developed science in human psychology, studying personality and specific personality traits are not without their problems. Reported dangers include the use of circular arguments, or 'reification' (Allport, 1973). Skinner (1953) for example, referred to the characterisation of a man as quick to anger because he may fight as, "*merely redundant descriptions*", because it is a character projected on the man from observing him fight. A second criticism, is that people can exhibit different tendencies in different situations, 'situationism' (Allport, 1973), with the importance of experience and present circumstances (such as education level, marital status and age) overriding the influence of personality. For example, someone who may not be aggressive at home, or in work, may be extremely aggressive in a competitive sporting situation. Distinctions have also been made in the human literature between provoked and unprovoked aggression (e.g. Hubbard et al. 2001). The same principles may apply to animals; for example, an animal might become extremely aggressive in situations where it is also fearful and perceives a threat, but not at other times. Similarly, female mice generally only attack intruder mice when they have young to protect (Benus, 2001). In applying personality studies to the analysis of aggression, it is vital to gain a very detailed understanding of aggressive trait-behaviour across multiple situations. This may help to avoid the problems highlighted in human psychology; including premature conclusions of situation specific aggressive behaviour as a trait aggression, and disregarding the impact of other personality traits that conflict with, or mask, aggressiveness.

1.1.3 Aggression and aggressiveness in social groups

In many species stability in social groups is maintained through a social order, where dominance relationships are formed and maintained through agonistic encounters (or the threat of such encounters). It is likely that there is some influence of aggression on dominance, although having a high rank within a social group does not always equate to being the most aggressive (e.g. Erhard & Mendl, 1997), with other factors such as age, weight, and parentage having an influence. Stability in a social group is considered beneficial for those within the group (Mendl & Held, 2001); however, individuals differ in whether they accept, or constantly challenge their social rank, which may consequently result in greater stress and pathologies associated with chronically high HPA reactivity (Sapolsky, 1990; Mendl et al, 1992; Ruis et al, 2001). Studies of wild primates report that low ranking animals remain within the social groups that suppress them (Sapolsky, 1990), indicating that there must be some benefits to group living that outweigh the cost of having a lower social status. However, animals in captivity no longer have the option to leave when the costs outweigh the benefits (Mendl & Deag, 1995). In the crowded conditions of commercial farming, this motivation to maintain optimal groups may be one factor in the occurrence of aggression, as the wild the ancestors of these animals would not exist in such high densities, or artificial groupings. Escalated aggression may therefore be the result of a desire to drive away unfamiliar animals (Puppe, 1998), or frustration at being unable to escape from such close proximity. Variation in aggressiveness may be a result of differences in coping abilities between animals that are more or less accepting of crowded conditions, and/or lower social status. There is some evidence, from a functional perspective, that these differing strategies offer divergent advantages to proactive/reactive copers in natural conditions. In mouse colonies, where dispersal is possible, a proactive style (SAL) is advantageous in stable colony groups, whereas reactive mice fare better when exploring and securing new territories (van Oortmerssen et al., 1985; Benus et al. 1991; Koolhaas et al., 1999).

1.1.3 Studies of aggression and aggressiveness in pigs

Various approaches have been used to study the occurrence of aggression in pigs and to understand what aspects of the commercial system induce aggression, with the ultimate aim of eliminating or reducing it (see Petherick & Blackshaw, 1987; Arey & Edwards, 1998; Marchant-Forde & Marchant-Forde, 2005). This review concentrates on aspects of the social

environment, although as mentioned already, the physical environmental can also impact upon levels of aggression. Attempts have been made to imitate aspects of natural social conditions, by for example, allowing piglets to meet other litters before weaning (Donaldson et al., 2002; D'Eath, 2005; Parratt et al., 2006). Some authors have tried to predict when particular pigs would be expected to fight by theorising the relative costs and benefits to those involved, using variables such as assessments of relative fighting ability (Jensen & Yngvesson, 1998), or resource holding potential (RHP). Assessment of RHP may come from physical characteristics, such as body weight, but may also be influenced by recent experiences of success or defeat (Van Doorn et al., 2003). These models often assume however, that animals can switch between tactics (mixed strategy) based upon their opponent's RHP or some physical attribute, e.g. if larger then be aggressive (Crowley, 2000; Switzer et al., 2001). Whilst physical and learnt characteristics will probably alter the likelihood that a pig will behave aggressively, in recent years the variability between individuals that cannot be explained by RHP alone has received more attention.

Studies of coping strategies, which categorise animals as active/proactive or passive/reactive, have been popular in pig research on individual differences. The main reason for this being that one of the major determining factors of coping style rests upon the propensity of an individual to be aggressive towards an unfamiliar conspecific and also that this tendency appears to be heritable (Benus et al., 1991). If these same coping styles were found in pigs, there would, therefore, be the potential to select against aggressiveness. Coping styles have been explored widely in pigs (e.g. Hessing et al., 1993; Jensen, 1995; Ruis et al., 2000; D'Eath & Burn, 2002; Janczak et al., 2003a; Bolhuis et al., 2005), but opinions differ about whether these dichotomous coping patterns can be found in pigs and also whether a true dichotomy of high/low aggressiveness occurs (as opposed to continuous variation). Hessing et al. (1993) were the first to propose that pigs could be categorised using similar coping strategies to those in the rodent literature. They found that pigs that showed high resistance to being placed in a supine position (back test) were more active, more likely to show escape behaviour and did not investigate a novel object thoroughly; whereas, non-resistant pigs were more hesitant but explored a novel object more thoroughly. Studies have also reported differences in physiological measures, such as heart rate (Hessing et al, 1994b) and HPA reactivity (Ruis et al, 1997). It has been proposed that stable and consistent reaction patterns exist in both social and non-social contexts (Ruis et al, 2000; Thodberg et al, 1999), which can be predictive of behaviour in unrelated circumstances (Erhard et al, 1999). This does however, contradict the findings of several others (Lawrence et al, 1991; Jensen, 1994;

Jensen et al, 1995; Forkman et al, 1995; Spoolder et al, 1996). Even intra-test (similar or same situation) consistency varies between those that found some consistency (Lawrence et al, 1991; Spoolder et al, 1996; Hessing et al 1993; Erhard and Mendl, 1997), and those that found little or none (Forkman et al, 1995; Jensen et al, 1995). This has led to debate over the validity of coping styles in pig research. There do not appear to be the same clear, measurable and repeatable differences in pigs as have been found in rodents. It may be therefore that this theory is too simple to explain the variation seen in pigs.

In more recent years behavioural researchers have been drawing parallels with the more advanced study of individual differences in humans. This has led to a change in the terminology with terms such as temperament (Manteca & Deag, 1993), personality (Gosling & John, 1999) and behavioural syndromes (Bell, 2007) being used. There is some evidence for an aggressiveness temperament, or personality, trait in pigs: several authors have reported observable differences between individuals in levels of aggressiveness (Benus et al., 1992; Erhard et al., 1997); there is evidence for consistency of responses over short periods of time within individuals and situations (Erhard & Mendl, 1997; D'Eath, 2004); and some predictability across different social challenge situations (Hessing et al., 1993; D'Eath, 2002). To understand trait aggression as a part of personality in pigs then, in addition to demonstrating the characteristics of a trait, there should be associations between divergent situations that correspond to groups of linked traits. This is a similar approach (albeit more flexible) to that of coping style theory, in that aggression may be linked to other behavioural traits such (e.g. boldness) and also to physiological characteristics (e.g. sympathetic-adrenomedullary reactivity). If aggression were found to be not only a trait, but also part of a much larger group of traits that make up personality, then it may consequently become undesirable to select against it, if this selection then resulted in removing associated positive behavioural traits (e.g. good maternal behaviour). Additionally, aggression may be impossible to select against if it is central to pig behaviour, e.g. to form social groups bonds (Erhard & Schouten, 2001). The fundamental nature of aggression may be one explanation for the lack of success in breeding for low aggression mice, compared to the high success rate for breeding very aggressive mice (van Oortmerssen & Bakker, 1981). If links between aggressiveness and positive traits were found, then this would impact upon the future direction of aggression and welfare studies in pigs, as attention must then be devoted to reducing excessive aggression through environmental rather than genetic manipulations.

To determine whether aggressive behaviour can be considered a trait of individuals, pigs should be studied at numerous points throughout their lives to look at the prevalence of aggression and consistency within and between situations. To gain a better understanding of how aggressiveness might function as a part of personality, then additional information should also be gathered about the overall way in which these animals behave in both social and non-social contexts. This would include measures related to, but not directly correlated with aggression, such as social status; but should in addition, include measures that are not obviously linked to aggression and that could also be trait-mediated as part of personality (such as maternal ability or infanticide). Studies of personality traits in pigs are still in their infancy. Nonetheless, this may be a promising approach that could help to explain why after many years of aggression studies in pigs there is still no real consensus of opinion on how (or even if it is possible) to solve the welfare problem of pig aggression in intensive systems. Perhaps previous studies have tried to find simple relationships where they do not exist, as aggressiveness is likely to be influences by many factors, such as the other traits that form part of personality, but also other situation specific factors. This thesis will concentrate on understanding aggressiveness as a trait of individuals, specifically how stable it is within individuals, over time and across situations. Additionally, there will some investigation of other trait-behaviours, to demonstrate that studies of aggressiveness should not be considered in isolation from other traits.

1.2 Objectives

The objectives of this thesis were to understand whether relative aggression in response to repeated social challenge was a constant characteristic of individual pigs, and if age or experience altered the quantitative or qualitative expression of this aggressive trait. Additionally, whether RIT aggressiveness was comparable to, or predictive of, aggressiveness during social mixing, and the different strategies adopted by pigs of differing aggressiveness during mixing. Finally, if aggressiveness was linked to responses to a challenging environment that was unconnected to social confrontation (maternal behaviour). A cohort of commercially reared pigs was followed from birth to slaughter, during which time they underwent repeated social confrontation tests to look for evidence of consistency within individuals, within situations and repeatability across similar situations. In addition, the patterns of behaviour displayed by aggressive pigs and their non-aggressive counterparts during mixing were examined for differences in fighting success, injuries suffered, social

rank obtained and relative stress responses (using cortisol levels as a physiological indicator of stress). In a subset of female pigs comparisons were made between these measures from social confrontations with the expression of maternal behaviour. This was to gain a better representation of how aggression might integrate with other behaviour traits that make up personality; maternal behaviour pre and post parturition was chosen as this behaviour has important welfare and production consequences.

1.3 The Study

The study took place between 2003 and 2005 at Easter Howgate Pig Unit (EHPU, Scottish Agricultural College, Edinburgh) and followed the development of a group of pigs from birth, with particular experiments encompassing various life-stages. Detailed below is a description of standard practice at the pig unit, followed by an overview of the entire study and finally a summary of each of the experimental Chapters.

1.3.1 EHPU – standard practice

The unit was run as a commercial indoor piggery, with additional emphasis on welfare; for example, all pigs had access to straw and piglets were not teeth-clipped or tail-docked. To maximise the practical relevance of any findings, the experimental pigs underwent the same husbandry procedures and were housed in the same buildings as the commercially reared pigs throughout the entire experiment. All pens were cleaned daily, apart from sow pens, which were cleaned twice-weekly. Pigs were fed ad libitum at all stages, with the exception of non-lactating gilts[1] and sows, which were feed-restricted to maintain their weight and received one meal each morning. Lactating sows were fed twice daily. Sows were moved into standard farrowing crates three to five days before their predicted farrowing date and remained there with their litters until weaning (approximately 28 days after birth). Piglets were tagged and weighed within 24 hours of birth and given iron injections three days post birth. The commercially reared piglets received no other interventions until weaning, whereas experimental piglets were weighed and involved in testing (e.g. Stock-person directed aggression test, Chapter 5) at several points. Weaning took place in the mornings.

[1] Throughout this thesis the term 'gilt' refers to female breeding pigs from puberty up to the birth and weaning of their first litters, from which point onwards they are termed sows.

The sow was first removed from the pen and then the piglets were weighed and given new ear-tags. Piglets were then transferred to weaning pens where they were mixed with other weaned litters. Standard practice at EHPU was to mix piglets from multiple (up to six) litters at weaning, making equal sized groups of between 15 and 20 pigs. When the smallest pigs in each weaned group reached 20 kg the group was moved from weaning pens to grower accommodation and at 40 kg, to finisher accommodation. Pigs were sent for slaughter when they reached approximately 90 to 110 kg live-weight. Pigs were kept at commercial or lower stocking densities, with a maximum of 20 growing pigs and six gilts/sows per pen. EHPU used a weekly-batch-farrowing system, whereby sows were housed in groups of six, with the aim of inseminating and then farrowing all six at the same time. Each room in the farrowing house contained six farrowing crates, with one group/room farrowing each week and one group/room weaned each week. These sows could then all be returned to the same social group. However, it was rarely the case that all six sows farrowed at the same time, due to problems such as asynchronous oestrous, or some sows not becoming pregnant (returning service). Therefore sows were usually mixed into groups with some unfamiliar sows post weaning of their litters.

1.3.2 Overview of study

The experimental animals were the progeny of 19 gilts, followed from birth until they reached slaughter weight and then for a group of selected females to the birth of their first litter (Figure 1.1). There were originally 24 gilts, however four of these pigs did not produce litters within the time period required due to health and fertility problems. One of the gilts died shortly after farrowing and so that litter was also excluded. The gilts had been artificially inseminated with single-boar, dam-line (breeding line) semen from one of two boars (ratio of litters per boar 10:9). Litters were born over a six-month period. The gilts were the daughters of pigs used in an experiment on prenatal stress (see Jarvis et al., 2006). Seven of the gilts had been born to gilts 'stressed' in the second trimester of pregnancy (by mixing with unfamiliar sows), seven were born to gilts stressed in the third trimester and five had been born to gilts that had not been stressed during pregnancy. These treatments are not discussed in the thesis as no effects were found between experimental pigs of 'stressed' and 'unstressed' grandmothers.

In this study pairs of litters were mixed at weaning (Chapter 3). To mimic commercial practice and to avoid selection of animals with particular characteristics (e.g. heaviest) there

was no selection for a certain number of piglets. All healthy piglets were mixed and because of this group sizes differed, although the two litters to be mixed together were matched for age and litter size as closely as possible. As five gilts did not produce litters within the specified time period five litters were without a mix-pair. Three farm-pig litters were used as substitutes and two large experimental litters were split in half, with each half mixed separately. The farm-litters were matched to the experimental litters by litter-size and age. The only difference between the farm and experimental litters was that the former had been born to multiparous sows served with terminal line (meat-line) semen. After mixing at weaning the pigs remained in the same social groups, but were moved at the appropriate points into increasingly bigger pens, from weaning to grower pens and then to finisher pens. At the beginning and end of the growing phase and prior to slaughter, pigs underwent Resident Intruder Testing (RIT) (Chapter 2). This was to test the reaction of individual pigs to a smaller unfamiliar pig that was introduced into a section of the home pen. The test was terminated after either pig attacked, or after five minutes if there was no attack. Pigs were assigned to treatment groups that determined how many RIT they would experience (Figure 1.1). Prior to slaughter the pigs were also tested in a group-feeding competition test to obtain a measure of relative social rank.

To examine the behaviour of pubescent and post-pubescent gilts 36 females were selected to enter the breeding herd. Selection was based on health status, leg conformation and similarity of testing experience. Six groups of six gilts were formed. The selected 36 gilts represented 11 out of the 12 weaning groups and 16 of the 19 original experimental litters. Four groups were mixed (Chapter 4) and consisted of three pairs of sisters, each pair from a different litter and weaning group (24 gilts from 12 litters and nine weaned groups). Two groups were not mixed as gilts and contained three sisters from each of two litters mixed at weaning (12 gilts from four litters and two weaned groups). These females were therefore familiar to all the other pigs in the gilt group and related to half of the group. Gilts were housed in their groups of six in the finisher accommodation until they reached a suitable size to move into sow housing. Between 40 and 45 days after the formation of the gilt groups the 36 females underwent another RIT.

Asynchronous oestrus within groups was a common problem in primiparous gilts on the unit and so to increase the probability that all of the gilts within a group would farrow at the same time altrenogest (Regumate) was administered. Where possible the occurrence of oestrus was recorded and gilts were served on the third oestrus post sexual maturity using artificial

insemination with terminal line semen. Pigs that did not subsequently become pregnant were served during the following heat (three weeks later). Three gilts did not become pregnant after three attempts and were excluded from the experiment at this point. Another gilt was excluded from the data analysis in Chapter 5 (maternal behaviour) as it was discovered that she had become pregnant by a group-mate in the finisher accommodation. Of the 36 sows, 32 went on to produce litters. Farrowing was staggered over a 6-month period and various measures were taken, including the maternal behaviour of the gilts and their reaction to a stock-person handling their piglets (Chapter 5).

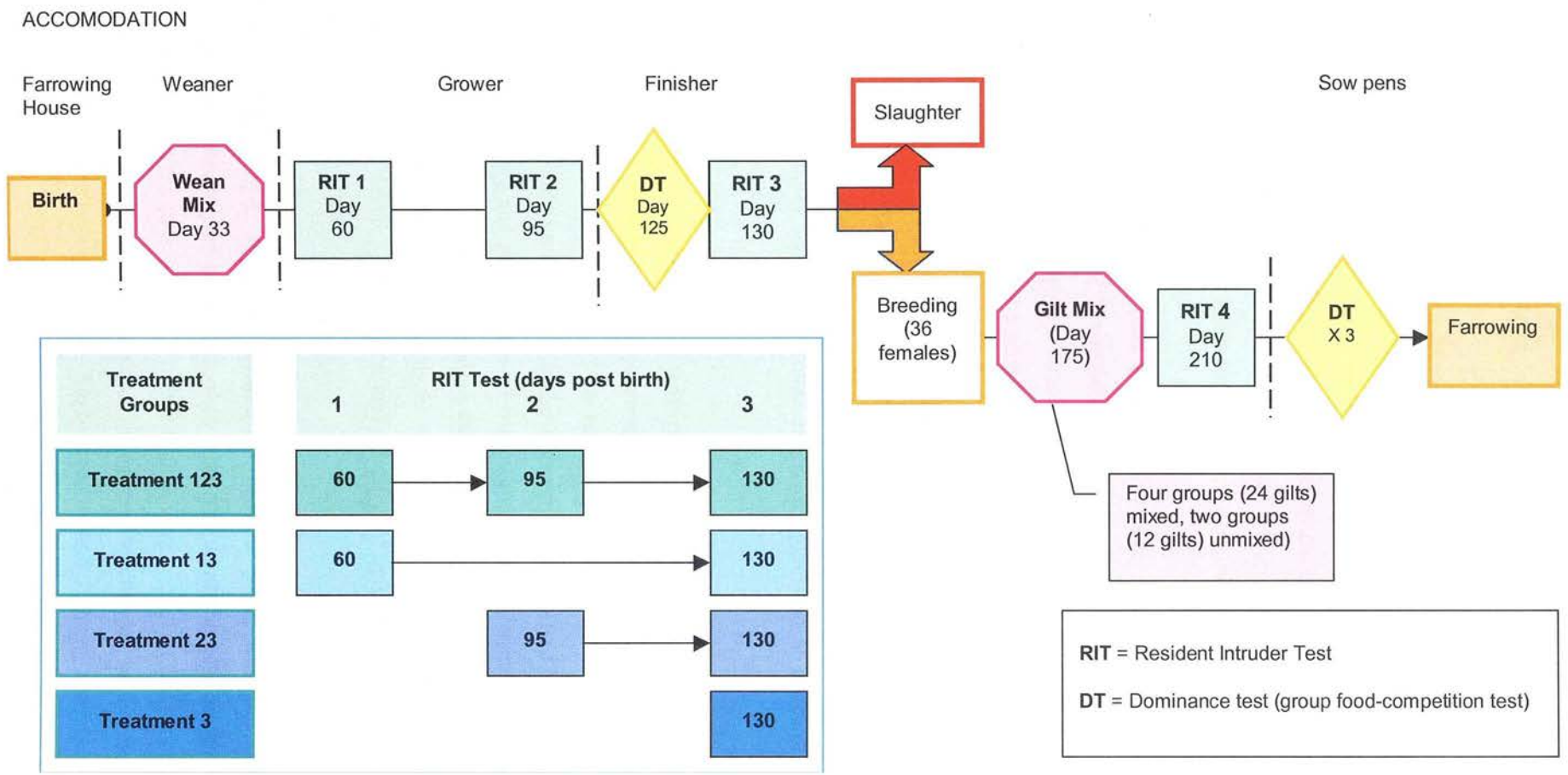


Figure 1.1 Lifeline of pigs detailing the test points and changes of accommodation, with a diagram indicating the sequence and number of resident intruder tests for each treatment group (T123, T13, T23 and T3)

1.3.3 Experimental chapters

The thesis is divided into four experimental Chapters. The first, Chapter 2, looks at the effect of age and experience on the propensity to be aggressive and whether consistency exists between tests of aggression. The remaining Chapters examine aggression at key stages during the life of commercially reared indoor pigs where aggressiveness may be a particular issue; mixing at weaning, mixing into breeding groups of gilts and the behaviour of primiparous gilts at farrowing (including aggression towards piglets).

1.3.3.1 Chapter 2, Do age and experience matter? Consistency of aggressive responses and the effect of repeated Resident Intruder Tests on pigs

This experiment examines aggressiveness measured over lifetime in growing pigs, using the Resident Intruder Test (RIT) as a social challenge. Pigs were presented with this same social challenge situation at three key stages in their life (post weaning, growing phase, pre-slaughter). Treatment groups underwent different combinations of the three tests, designed to account for any effects of age and test experience. Using the occurrence and speed of attacking, the consistency of responses and relative rank-order aggressiveness over time were investigated, as were the absolute levels of aggression at different ages and with differing test experience.

1.3.3.2 Chapter 3, Behavioural strategies during mixing at weaning in male and female pigs of differing aggressiveness; effects on fighting success, injuries received, dominance status and cortisol

This part of the study investigates whether pigs can be categorised by differing behavioural strategies at mixing and also whether such strategies confer differing success or social rank. A detailed ethogram was used to analyse the behaviour of all the pigs in 12 weaning mixes. The results of these behavioural analyses were compared to measures of fighting success, salivary cortisol levels, the number and severity of injuries received and social rank measured at a later date.

1.3.3.3 Chapter 4, Aggressiveness in female pigs, comparisons between behavioural strategies of female pigs mixed at weaning and as gilts

This experiment assesses whether the female pigs were consistent in aggressiveness across lifetime and if the structure and severity of aggression changed as the pigs matured and became more socially experienced. Aggressiveness and fighting success were measured

during mixing and using a Resident Intruder Test. Salivary cortisol and injuries sustained during mixing were also recorded. Social rank was measured using several food-competition tests. These data were then compared to data obtained for these same pigs during previous RIT (Chapter 2), mixing at weaning (Chapter 3) and also a dominance test performed on the mixed-sex weaned groups.

1.3.3.4 Chapter 5, Does having an aggressive personality effect maternal ability?

This chapter investigates whether gilts of differing aggressiveness and success during social encounters also differed in maternal ability and reaction to the farrowing environment. Parameters investigated included whether aggressive and/or successful pigs were more aggressive towards their own piglets or to stockmen. Also, if there were predictors of maternal behaviour from the ethogram of behaviours measured during previous social encounters with conspecifics. Behavioural measures included postures, nest-building pre and post farrowing, as well as gilt-piglet interactions (aggressive and non-aggressive). Salivary cortisol levels were analysed at various points pre and post farrowing. Data were then compared to the measures of mix-aggression and RIT latency to attack described in Chapter 3.

Finally, the findings from these various key stages in the lifetime of the study animals are discussed in Chapter 6.

CHAPTER 2

DO AGE AND EXPERIENCE MATTER? CONSISTENCY OF AGGRESSIVE RESPONSES AND THE EFFECT OF REPEATED RESIDENT INTRUDER TESTS ON PIGS

2.1. Introduction

Excessive aggression between pigs is an undesirable consequence of modern farming practices that detrimentally effects the health and welfare of the animals and consequently impacts producers financially (Tan et al., 1991; Mendl et al., 1992; Stookey & Gonyou, 1994). Pigs fight when they encounter unfamiliar animals to establish a social hierarchy or to drive strangers away (see Rushen, 1988; Puppe, 1998). In wild and feral pigs aggression is rare as they live in stable family groups, whereas most commercially reared pigs will undergo mixing with unfamiliar pigs at least once during their lifetime. This is a particular issue for breeding females, as they will often experience frequent mixing throughout their adult life.

Numerous environmental approaches have been tried to reduce aggression, (e.g. scent sprays, timing of mixing, altering groups size etc) with little success (Petherick & Blackshaw, 1987; Marchant-Forde & Marchant-Forde, 2005). An alternative approach has been to examine why some pigs are more aggressive than others when placed in the same situation. The frequency, duration and intensity of aggression often differ between groups of pigs and between individuals within a group (e.g. D'Eath, 2002; Bolhuis et al., 2005). If it can be demonstrated that certain animals are inherently more aggressive than others then this might provide a means to reduce the amount of fighting at mixing. This could be through selective breeding for example, or through changes to husbandry practices that either reduce the occurrence of mixing, or that use specific mixing strategies for particular combinations of aggressive-type individuals.

In order for aggression to be considered a trait it needs to meet certain criteria; these include definable differences between individuals that are repeatable across time in the same situation and predictable across similar situations. Assessing the consistency of aggression over successive mixing events is not straightforward. The level of aggression manifested is likely to be both a product of the individual animal and the combination of individuals being mixed (Erhard et al., 1997); so it is difficult to replicate exactly the same testing experience. In addition, the effect of learning must be considered, as with repeated exposure novelty is lessened and the situation may then evoke different motivations and responses. There is considerable evidence in both rodents and pigs that repeated mixing of unfamiliar animals changes the level of aggression and speed with which fights are resolved in subsequent social encounters (Parmigiani & Brain, 1983; van Putten & Bure, 1997; Giersing &

Andersson, 1998; D'Eath, 2005). The age at which animals experience aggression is also an issue worth considering. In many young animals periods of heightened sensitivity to external influences have been documented (Karsh & Turner, 1988). Similarly, relative aggressiveness may alter with time and maturity (Francis, 1990; Roberts & DelVecchio, 2000; van Beijsterveldt et al., 2003), particularly in young animals where changes can occur over relatively short periods of time (Pitts et al., 2000). This issue is important when considering selective breeding programmes based on aggressiveness at one point in development, as this may bear little relation to aggression at a later stage. It is often assumed that male and female pigs will not differ in their reactions to social challenge and this finding has been reported in several cases (Erhard & Mendl, 1997; D'Eath & Burn, 2002). Looking at the natural life history of pigs, the social organisation of the sexes diverges with the onset of puberty, with males leaving the family group. As growing pigs tend to be slaughtered before sexual maturity there is usually little distinction made between sexes in studies of aggressiveness. However in many species there is a difference between sexes in likelihood or speed of attacking an unfamiliar animal (e.g. Brain et al., 1980; Blanchard et al., 1984; Francis, 1990). In some cases the likelihood of attack is related to the physiological (e.g. hormonal) status of the attacker, such as during lactation (Lonstein et al., 2005), or is dependent on the sex of the opponent (Whalen & Johnson, 1987). It is possible that whilst differences may not be evident early in development, as pigs mature their reactions to unfamiliar animals in social encounters may become more sex-specific.

Much of the work on individual differences between pigs in aggressiveness has been done using a test adapted from studies of rodent aggression (see Kemble, 1993). The Resident Intruder Test (RIT) is a standardised test of aggression, where a resident pig, given the advantage of territory and size, has the opportunity to attack an unfamiliar pig. Aggressiveness is measured by whether the pig attacks or not, and if it does attack, how quickly it does so. Unlike mixing, the RIT is simplified by having one focal animal at a time, with all resident animals experiencing the same controlled procedure. Like mixing, the RIT is a social challenge where the focal pig is confronted by an unfamiliar animal. It has been suggested that the response to this test is independent of factors such as age and sex (Erhard et al., 1997), making it a good test of temperament. The test therefore has the potential to be used as a measure of consistency in aggressiveness over time.

As already mentioned learning from experience during repeated testing is an issue to consider. The RIT could be considered less severe and therefore less detrimental to welfare

than mixing. It also provides the test subject with less fighting experience, thus reducing the impact of learning. However, there are examples of RIT experience altering responses. In a study on the behaviour of aggressive and non-aggressive intruders, aggressive residents were trained to fight using several brief social confrontations (Benus et al., 1992). Aggressiveness and ability to win in subsequent conflicts was increased through confrontations lasting less than 30 seconds, where contenders were separated before a full fight, but after 2 attacks (one more than in the RIT). It was suggested that ending the conflict before resolution 'primes' the attacker, leaving it in an elevated state of aggression. This priming effect has also been reported in pigs, with attacks being faster on the second day of testing (Erhard & Mendl, 1997; D'Eath & Pickup, 2002; D'Eath, 2004).

This experiment examined aggressiveness over lifetime in growing pigs using the RIT. To demonstrate whether consistency exists in the likelihood of attacking, the time taken to attack and relative (rank-order) time to attack, male and female pigs were presented with the same social challenge situation at three key stages in their life (post weaning, growing phase, pre-slaughter). Pigs that attacked an intruder more frequently and more quickly were considered to be more aggressive. Treatment groups experienced different combinations of the three tests, designed to account for any effects of age at first test and differing test experience. The effect of recent as opposed to distant prior-experience was also examined, as were the differences between male and female pigs in attack latency. As differences between litters in absolute aggressiveness have been shown (Erhard & Mendl, 1997; D'Eath & Lawrence, 2004) treatments were balanced across litter. Measures from the RIT were also compared to a measure of aggression from mixing post-weaning. This was to examine whether the pigs that attacked more frequently in the period immediately following mixing into new social groups with numerous unfamiliar pigs were also the fastest to attack, or more frequent attackers, when exposed to a single unfamiliar pig in the RIT.

2.2 Methods

2.2.1 Animals and housing

The subjects were 163 (78 male 85 female) Large White x Landrace pigs born over a six-month period to 19 primiparous sows of similar history. The sows were served by artificial insemination with semen from one of two dam-line (breeding stock) boars (semen supplier PIC, Sygen). Pairs of litters, of similar age and group size, were mixed together at weaning to form 12 wean-mix groups (mean group size 16.7 ± 3.9 SD). On five occasions there were insufficient litters of the correct age. This problem was solved on two occasions by dividing two very large litters, so that each 'half' was mixed into a new group. So that the litter groups mixed together were of similar size (to avoid one litter having an advantage over the other) the larger litters were not divided into equal halves; the smaller subsets were mixed with two very small litters (each containing no more than seven pigs). The three remaining unpaired litters were mixed with litters of the same age and group size, born to multiparous farm sows served with terminal-line (meat stock) semen. These meat-pig litters were not tested.

The sows and their litters were kept in a standard farrowing crate system (pen dimensions 1.50 x 3.14 m). Pens had concrete floors with a slatted dunging area at the rear and a floor-heated piglet kennel (0.54 x 2.22 x height 1.05 m, temperature 30 °C) at the sow-head-end. The floor of the crate was covered with wood-shavings and some straw. Each crate had a raised food trough and nipple water drinker for the sow. There was a nipple water drinker just above floor level for the piglets. The room temperature was maintained at 21 °C up to seven days post partum, and then at 18 °C until weaning. Piglets were identity tagged within 24 hours of birth using plastic ear tags; these tags were replaced with larger ones at weaning. Iron injections were administered to the piglets at three days old. Teeth were not clipped and tails were not docked. Sows were provided with up to 5 kg of feed morning and evening (Scotlean Lactating Sow Pellets, ABN). The piglets were offered approximately 0.2 kg of dry feed (Scotlean Silver Pellets, ABN) from 18 days old, distributed on the creep floor in the morning.

Piglets were mixed on the morning of weaning. Weaning took place after day 27 post-partum (mean age 33.74 ± 3.4 SD days). To ensure that individuals could be identified each piglet was numbered and given a litter-specific mark using stock marker. They were then moved

immediately from the farrowing crate to a weaning pen, youngest litter first. Weaning pens contained a straw-bedded unheated kennel, an uncovered sloping floor area leading to the dunging area, a water drinker with three nipple dispensers and a feeder trough with four spaces (feedface length 0.9 m, pen 1.75 x 2.05 m, kennel 1.25 x 1.75 m). When the smallest individuals in the group reached a minimum of 20 kg they were moved to grower accommodation. Grower pens consisted of a straw area with a feeder (four-space feeder, feedface length 0.9 m), and a dunging passage with 2 drinkers (pen 1.8 x 5.0 m, dunging passage 1.8 x 1.8 m). Groups were moved to larger, finisher accommodation, of the same design as the grower pens (three-space feeder, length 0.9m, pen 2.35 x 6.00 m, of which dunging passage 2.35 x 2.35 m) when the smallest animals reached 40 kg. Groups were weighed weekly in the weaning accommodation. When in the grower and finisher pens, groups were weighed on the days prior to the Resident Intruder Tests.

The pigs underwent the same husbandry practices and occupied the same buildings as non-experimental farm animals at all stages. The temperature and ventilation inside the buildings were controlled by fans (farrowing house only) or automatically controlled vents (natural ventilation). Artificial light was used in all buildings, from 0730 to 1600 hours, although the automated vents and windows meant that some natural light was also present. Pens were cleaned every morning and fresh straw was provided in all accommodation. Pigs were fed ad libitum with standard commercial feed (Scotlean Prime Link, LC Rearer and Finisher Pellets, ABN).

2.2.2 Treatments

The treatment group each pig was assigned to determined the number of Resident Intruder Tests (RIT) it would receive (Figure 2.1). There were three testing points that corresponded with specific points in the life of growing pigs. The first was at 60 days old (RIT 1), shortly after the pigs were moved into grower accommodation (after allowing for an acclimatisation period of at least a week). The second test, at 95 days old (RIT 2), was performed just prior to the pigs being moved into finisher accommodation. The last test was performed before slaughter at 130 days old (RIT 3). Pigs assigned to the treatment group receiving tests at all points were referred to as T123, those receiving tests 1 and 3 as T13, tests 2 and 3 as T23 and pigs only experiencing test 3 as T3.

To compare pigs of differing mix-aggression four pigs per litter were selected based on their relative aggressiveness during the initial 30 minute period post-mixing at weaning. The numbers of attacks were recorded by direct observation; attacks were classed as head-knocks, single bites, or a series of bites directed to another pig. The highest and lowest attacking male and female of each litter were preferentially allocated to the treatment group tested at all points (T123); if these pigs were not suitable, due to illness for example, then the next highest/lowest were chosen. Where there were equally high/low aggression animals the choice was made at random between them. The rest of the litter was allocated randomly to the remaining treatments, balanced for sex. Of the 177 pigs weaned, 16 died before the end of the experiment (this was due to Postweaning Multisystemic Wasting Syndrome (PMWS) in most cases).

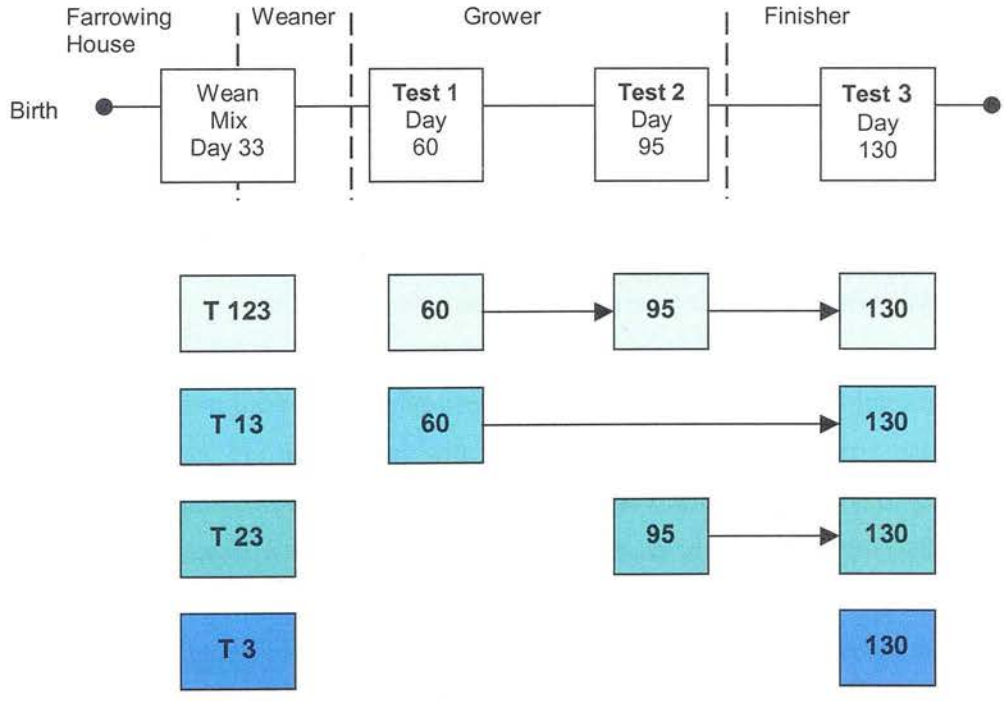


Figure 2.1 Lifeline of pigs detailing the test points and changes of accommodation, with a diagram indicating the sequence of RITs for each treatment group (T123, T13, T23 and T3)

2.2.3 Resident Intruder Test (RIT)

The resident intruder tests began at approximately 1030 hours, after the animal houses had been cleaned. Most tests were completed by 1300 hours and no tests were started in the afternoon. The methods for this test have been described in detail in Erhard & Mendl (1997) and D'Eath & Pickup (2002). In summary, the 'resident' was isolated from its social group in a section of the resident's home pen (grower pens 1.80 x 1.65 m, finisher pens 2.35 x 1.80 m), using a solid partition. A smaller (younger) intruder pig (72.7 % ± 5.1 SD the weight of the resident), unfamiliar to the resident, was introduced into the test arena. Intruders were from different litters to the residents and no resident and intruder met on more than one occasion. Intruders were kept in the same buildings and conditions as the resident pigs. Pigs could be used as intruders on up to 12 occasions in their lifetime, although re-use was minimised and resident pigs were never used as intruders. A timer was started after the shoulders of the intruder pig entered the pen and the time the resident took to make initial contact (nose to body) was noted. The test was terminated when either pig attacked (rapid and persistent biting, with no less than four bites in four seconds), or where there was no attack (timed-out), five minutes from the resident making initial contact with the intruder. Once either end-point was reached, the pigs were immediately separated and returned to their groups. The test would also have been terminated if the resident had made no initial contact within 10 minutes of the intruder entering, although this never happened. Measures recorded were the test outcome (resident attack or no-attack) and latency to attack from initial contact. For tests ending with no resident attack (timed-out tests and intruder-attack), latency to attack was recorded as 300 seconds, the maximum duration of the test. Mounting behaviour was also recorded as it became clear early in testing that pigs showing mounting behaviour were unlikely to attack within the five minute test period. As the pigs grew heavier the mounted pigs ran the risk of being injured, particularly where it was the smaller intruder being mounted. Where mounting was severe and repeated (more than five attempts to mount and/or mounted animal becoming distressed or at risk of injury) tests were terminated early to protect the welfare of the pigs. The outcome of tests terminated early through mounting were also classed as no-attack and assigned a maximum latency of 300 seconds.

At each of the three testing points pigs were tested twice, on consecutive days, with a different intruder on each occasion. So each test point was actually a pair of tests (a and b), thus pigs in treatment 123 experienced the test at three points, but six times in total.

2.2.4 Statistical analyses

Results are presented in the form of yes/no data (1, 0) for the occurrence of attacks. Latencies to attack (seconds) are used for comparisons of absolute aggressiveness (speed of attacking) and consistency of rank order aggressiveness (relative speed to attack). Attack latencies were normally distributed; however, pigs that did not attack were assigned the maximum attack latency of 300 seconds. This censoring produced a second peak in the distribution curve. Data that included the non-attackers did not therefore meet the requirements of parametric statistics and non-parametric equivalents have been used for the majority of analyses.

Comparisons 'within test' refers to analysis of the individual tests performed on consecutive days at each time point: so analysis within test point 1 compared 1a & 1b; test point 2, 2a & 2b; test point 3, 3a & 3b. 'Between test' comparisons refer to differences between time points: between 1, 2 and 3, test days 60, 95 and 130 respectively. For the between test comparisons, latencies and binary data (0, 1, 2) were summed for a and b, unless otherwise stated. Overall comparisons of attack frequency, speed and consistency used data from treatment 123 (tested at all points). To test for the effect of experience, latencies at test 3 (day 130) were compared between treatments with two (T123), one (T13 and T23), and no prior experience (T3). To examine the difference between naïve pigs tested for the first time at different ages, latencies were compared for treatments 123 and 13 at day 60, with T23 at day 95 and T3 at day 130. To examine the effects of age at first testing on subsequent tests, the differences between latencies in the last test (day 130) were compared between early first experience (T123 and T13) and late first experience pigs (T23 and T3). To test for the effect of recent versus distant experience on day 130, latencies were compared for pigs with previous experience on either day 95 (T23), or day 60 (T13).

To test for differences in aggressiveness between sexes and differences in latency between naïve pigs at first testing (unpaired), latencies were compared using the Mann Whitney U test (U). Wilcoxon's Signed-Ranks (T) test for paired data was used for comparisons within test (between a and b) and between tests within treatment. Where comparisons of more than two tests where required the Friedman (Fr) test of repeated measures was used for within treatment comparisons and Kruskal-Wallis (H) (non-parametric equivalent to one-way ANOVA) was used for unpaired data.

Kendall's coefficient of concordance (W) was used to assess the level of agreement between all tests in treatment 123. Spearman's Rank Order correlation (r_s) was used to compare consistency in relative aggressiveness within and between tests.

It is possible that the level of rank order consistency, measured by the correlation coefficient, was affected by particular categories of pigs being consistently more or less aggressive than others. For example, if females had been consistently more aggressive than males then they would tend to score lower ranks and the correlation coefficient might therefore reflect the consistency of females compared to males and not individual consistency. To account for this, Spearman's correlation was run on the residuals of a Residual Maximum Likelihood (REML) model. Factors fitted were litter (random), resident weight and intruder weight as a proportion of resident weight (covariates), resident sex, sex of the intruder, treatment, sire, group, relative aggression in the wean mix and interactions between sex, with resident weight, group and treatment. The same model was run for each test point (1a, 1b, etc). Correlating residuals should give a better indication of individual consistency in relative aggressiveness after extraneous factors have been accounted for. A decrease in residual correlation, compared to the raw data coefficient, would indicate that factors other than an individual's consistency were previously explaining the coefficient. An increase would suggest that inconsistencies between categories (e.g. sex) of animal were masking the actual consistency of individuals. Data were transformed (log (attack latency+1)); but due to the peak of responses at 300 seconds the data were still non-normal, so the results of this test should be treated with caution. For this reason only the residual correlation values have been reported, not the REML test statistic or levels of significance. To analyse consistency between tests, residuals for each set of test pairs (a and b) were summed. Residual plots generally produced slightly more conservative correlation coefficients than those obtained from the raw data, except for comparisons across tests 1 and 3. The coefficients for both the residuals and raw data are presented, but only the residual analyses are discussed in the text (these generally gave more conservative coefficient values).

Chi-square (χ) was used as a test of association indicating consistency of attacking (1) or not-attacking (0), within test (2 x 2 design) and between tests (3 x 3 design, summed scores for test pairs being 0, 1 or 2). Where sample sizes were small, or expected counts < 5, Fisher's Exact test was used instead. This gives an exact probability and no test statistic. The consistency of mounting was treated in the same way.

Links between the sex of resident or intruder and occurrence of attacks or mounting were analysed using Chi-square or Fishers Exact test. For analyses including mounting as a category, all pigs observed to mount were classed as 'mounters', regardless of whether the test ended in an attack, timed-out or was terminated early. Both sexes were included, although the majority of pigs mounting were male. Chi-square was also used to test for any association between aggressiveness in the wean mix and attacking or mounting in RITs. Wean mix aggressiveness was calculated by counting the number of attacks initiated by each pig in the first 30 minutes post mixing and dividing the subsequent scores into quartiles; low aggressiveness (< Q1), moderately aggressive (Q1 - Q3) and highly aggressive (Q >3).

All analyses were performed using Genstat 8.0 (VSN International Ltd), except Chi and Fishers analyses, which were performed using Minitab 14 (2 x 2 contingency tables) and Vassar (http://vassun.vassar.edu/~lowry/VassarStats.html) for 3 x 3 tables.

2.3 Results

2.3.1 Overall effects

2.3.1.1 Does the occurrence of attacks and speed of attacking change over time?

Of the 778 tests performed, 64 % ended with an attack by the resident, 4 % ended with an attack by the intruder. The overall frequency of resident attacks declined with time (tests 1 - 3, 71 %, 68 % and 57 %), consistent with an increase in attack latency (Table 2.1, summed latencies (seconds), 1 = 282.5, 2 = 261.0, 3 = 366.0), as tests that did not end in an attack were given the maximum latency (300 seconds). Pigs were faster to attack on the second day of each test pair (median latency test 1a = 115.5s, 1b = 63.0s, T^{+} = 1436.0 $p < 0.001$; 2a = 107.5s, 2b = 85.5s, not significant $p = 0.1$; 3a = 233.0s, 3b = 158.0s, T^{+} = 2530.0 $p < 0.05$).

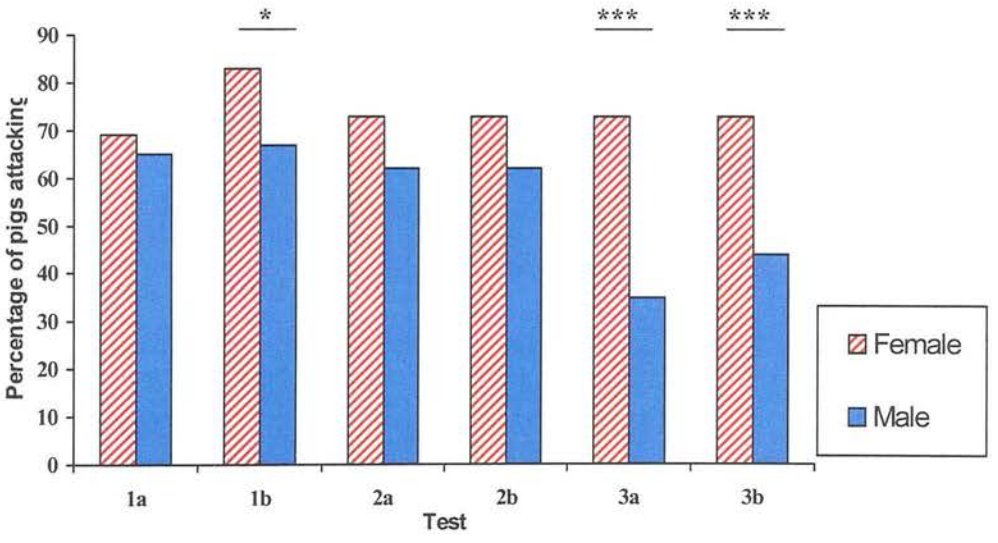


Figure 2.2 Percentage of females and males attacking at each test point, with levels of significance indicating differences between the sexes

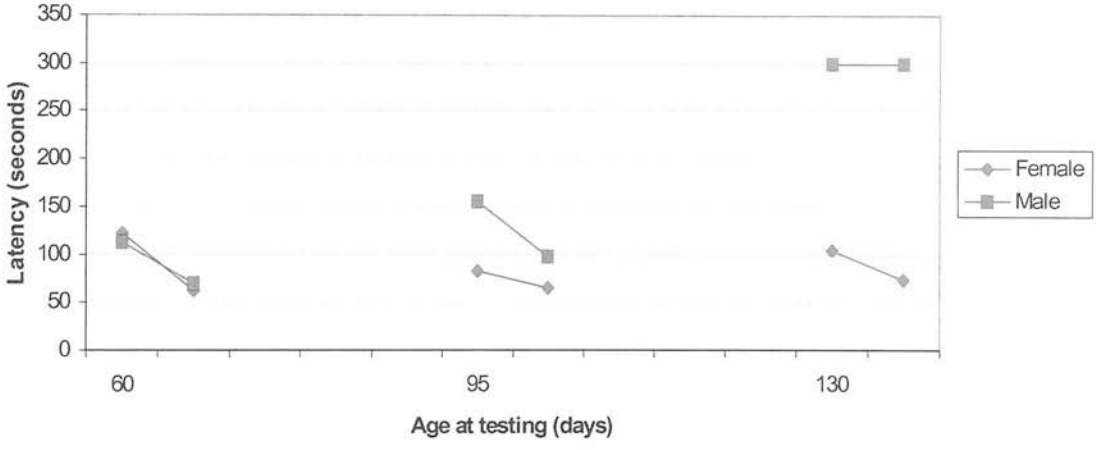
Examining the treatment tested at all points (T123), latency to attack changed over time (Fr_5 = 15.67, p = 0.003), with pigs taking longer to attack in the last test (Table 2.1). Probability of attacking was greatest in test 1b and remained high until test point 3 (Back-transformed means for attacking, test 1a = 0.68, 1b = 0.80, 2a = 0.74, 2b = 0.70, 3a = 0.61, 3b = 0.65, Wald $statistic_5$ = 11.25, p = 0.047).

Table 2.1 Median attack latencies (seconds), by sex and treatment, with sample sizes

Sex	Treatment	Median Attack Latency (seconds)											
		Test day 60				Test day 95				Test day 130			
		1a	1b	1 sum	N	2a	2b	2 sum	N	3a	3b	3 sum	N
Female + Male	All	115.5	63.0	282.5	112	107.5	85.5	261.0	114	233.0	158.0	366.0	163
Female	All	122.0	62.0	236.0	64	82.5	65.0	198.0	62	104.0	74.0	257.0	85
Male	All	112.5	70.0	314.5	48	155.0	98.0	331.0	52	300.0	300.0	540.0	78
Female + Male	123	131.5	63.0	295.0	90	101.0	69.5	261.0	90	174.5	111.0	345.0	90
	13	70.5	63.5	244.0	22	-	-	-	-	145.0	193.0	331.5	22
	23	-	-	-	-	174.0	125.5	259.0	24	300.0	250.0	461.0	24
	3	-	-	-	-	-	-	-	-	300.0	202.0	393.0	27
Female	123	148.0	63.0	292.0	53	55.0	54.0	162.0	53	84.0	68.0	167.0	53
	13	106.6	61.0	198.0	11	-	-	-	-	77.0	122.0	310.0	11
	23	-	-	-	-	255.0	144.0	444.0	9	295.0	100.0	371.0	9
	3	-	-	-	-	-	-	-	-	158.5	25.5	308.0	12
Male	123	111.0	142.9	320.0	37	187.0	110.0	339.0	37	300.0	300.0	459.0	37
	13	151.0	66.0	309.0	11	-	-	-	-	300.0	300.0	600.0	11
	23	-	-	-	-	91.0	86.0	182.0	15	300.0	300.0	500.0	15
	3	-	-	-	-	-	-	-	-	300.0	300.0	600.0	15

2.3.1.2 Sex differences

Of the 90 pigs tested at all points (T123), 24 attacked in every test and of these, 19 were female. Females were generally more likely than males to attack from test 1 (Figure 2.2), with this difference being highly significant in the last test. The female pigs did not show the same increase in latency over time as indicated in the overall analysis with the sexes combined. Differences between sexes in latency to attack were apparent by test 2 (Figure 2.3), with males tending to take longer to attack ($U = 1309.5$, $p = 0.08$) and by test 3 the difference was clear ($U = 1848.5$, $p < 0.001$). Thus it was surmised that the male pigs were responsible for the overall increase in attack latency and so results are presented for sexes separately where appropriate.



	Range (seconds)					
	Day 60		Day 95		Day 130	
Sex	1a	1b	2a	2b	3a	3b
Female	46 - 300	30 - 133.5	22 - 300	23 - 300	35.8 - 300	23 - 300
Male	64 - 300	24 - 300	41 - 300	32 - 300	106 - 300	74 - 300

Figure 2.3 Median attack latency for males and females at paired tests 1a and 1b (age 60 days), 2a and 2b (age 95 days) and 3a and 3b (age 135 days), with table detailing interquartile range (all treatments)

The proportion of females attacking remained consistent over the course of the experiment (Table 2.2), with the exception of an increase in attackers in test 1b (1a 69 %, 1b 83 %, 2a to 3b, 73 %). The females were slowest to attack in the first test (1a, 122.0s, 1b, 62.0s, $N = 57$, $T^{+} = 476.0$, $p = 0.006$), but after this median attack time was fairly consistent across the study (Table 2.1). Although latencies tended to be shorter on the second day of each test pair, this difference was not significant.

Table 2.2 Percentage of female and male pigs consistent in attacking or never attacking within-test (tests a & b on consecutive days)

Sex	Test	Attack Frequency (%) Never	Always
Female	1	11	63
	2	16	61
	3	15	61
Male	1	19	50
	2	25	48
	3	46	24

Males pigs took longer to attack in the final test, compared to earlier tests (summed tests, $H_2 = 15.82$, $p < 0.001$). There was a corresponding decline in attack-frequency (attacks, test 1 = 66 %, 2 = 62 %, 3 = 39 %, $\chi_4 = 15.207$, $p < 0.01$). Although there was a reduction in time to attack on the second day of test pairs (Figure 2.3), this only tended towards significance (1a & b, $p = 0.07$; 2a & b, not significant; 3a & b, $p = 0.09$).

Mounting was presumed to be the most likely cause of the increased attack latency to attack. At all points males were more likely to mount than females (incidence male:female, day 60, 30:4, day 95, 41:4, day 130, 72:6; significance 1a, $p = 0.002$, tests 1b to 3b, $p < 0.001$) with 45 females (53 %) and 8 males (10 %) never observed to mount. Mounting occurred less frequently than attacking, but pigs that mounted were consistent in doing so. Within test, there was a highly significant association, with pigs either mounting or not mounting on both days ($p < 0.001$). Mounting was also consistent between tests ($p < 0.001$). The overall proportion of pigs mounting in both tests of a pair remained consistent from a young age, with more pigs inconsistently mounting (in just one test of a pair) as they got older (Table 2.3).

Table 2.3 Percentage of pigs mounting within tests 1 to 3 (a & b) across all treatments

Test (a & b)	Not mounting in a or b	Mounted in both a & b	Mounted in either a or b
1	80	11	9
2	73	12	15
3	65	13	22

The sex of the intruders was less important. Overall female intruders were more likely to be mounted in only two of the six test pairs (2a, $\chi_1 = 5.28$, $p = 0.022$ 3a, $\chi_1 = 8.509$, $p = 0.004$), with a corresponding increase in the number of male intruders attacked (2a, 79 % males, 59 % female intruders, $\chi_1 = 5.27$, $p = 0.022$; 3a, 71 % males, 36 % females, $\chi_1 = 19.96$, $p < 0.001$). Analysed by the sex of the resident, males were less likely to attack female intruders in tests 2 and 3 (77 % males, 50 % females attacked, 2a, 50:77, $\chi_1 = 4.12$, $p = 0.042$; 2b, 50:83, $\chi_1 = 5.94$, $p = 0.015$, 3a, 19:53, $\chi_1 = 9.93$, $p = 0.002$, 3b, 32:55, $\chi_1 = 4.39$, $p = 0.036$); whereas female residents did not show a preference, except in test 3a, when they were more likely to attack male intruders (84 % males attacked, 57 % females, $\chi_1 = 7.48$, $p = 0.006$).

2.3.1.3 Weaning mix aggression

There was no correlation between speed of attacking in the RIT and the number of attacks in the first 30 minutes post mixing. In the first 30 minutes of the weaning mix male pigs initiated more attacks than females (median number of attacks, female = 3.0, male = 7.0, $U = 2193.3$, $p < 0.001$), so more males than females were classed as high mix-aggression pigs (proportion of males, high aggression 62 % intermediate, 53 %, low 24 %). There was an association between attacks during the first 30 minutes post mixing and the occurrence of attacking in RIT 1 ($\chi_4 = 14.02$, $p = 0.007$), but not in RIT 2 or 3. As expected, the low mix-aggression pigs were less likely to attack in RIT1; however, the high mix-aggression pigs also timed-out more than expected, and it was the moderately mix-aggressive pigs that attacked most frequently. When the sexes were analysed separately this relationship only remained significant in males ($\chi_4 = 15.21$, $p = 0.004$) and was not found in tests 2 and 3. Investigation of the association between attacking in the mix and mounting in the last RIT (3a, $\chi_4 = 8.22$, $p = 0.084$; 3b, $\chi_4 = 15.53$, $p = 0.004$) found that low mix-aggression pigs were unlikely to mount. Proportionally more mounters had come from the moderately mix-aggressive pigs. Although the high aggression pigs were male biased they did not mount

more than expected, even though they were less likely to attack. In summary, there was a sub-group of pigs that were not very aggressive in the mix or the RIT and were unlikely to mount. Moderately mix-aggressive were the most aggressive and the most likely to mount during the RIT.

2.3.1.4 Overall consistency of attacking and relative (rank-order) aggression

Within test there was a high degree of consistency in responses, with pigs being consistent attackers or non-attackers (1a & b, $\chi_1 = 10.57$, $p = 0.001$; 2a & b, $\chi_1 = 21.6$, $p < 0.001$; 3a & b, $\chi_1 = 36.49$, $p < 0.001$). This was still true when the sexes were analysed separately (1a & b, females, $N = 64$, $p = 0.026$, males $N = 48$, $p = 0.054$; 2a & b, females, $N = 62$, $p = 0.001$, males, $N = 52$, $p = 0.003$; 3a & b, females, $N = 85$, $p < 0.0005$, males, $N = 78$, $p = 0.0007$). There were a greater number of consistent attackers than consistent non-attackers within-test (Table 2.2), although this would be expected as attacking was the most frequent test outcome overall. However, this relationship was reversed in male pigs during test 3, where never attacking became more frequent than attacking. In general, a greater proportion of females than males consistently attacked and compared to female pigs, a greater proportion of males were consistent in not attacking.

Between tests there were again more pigs that attacked in every test compared to those that did not attack. There was also a greater proportion of females that were consistent attackers compared to the males, and more male non-attackers compared to the females. For the sexes combined, responses between test points were consistent, between days 60 and 95 (tests 1 and 2, $N = 90$, $p < 0.01$), 95 and 130 (tests 2 and 3, $N = 114$, $p < 0.001$), but not 60 and 130 (tests 1 and 3). Females showed slightly more consistency in responses between tests than males (days 60 and 95, females, $N = 53$, $p = 0.068$, males not significant; days 95 and 130, females, $N = 62$, $p = 0.001$, males, $N = 52$, $p = 0.01$; days 60 and 130, not significant).

Rank order aggressiveness (relative latency to attack) was consistent within test, both for the sexes combined and separately (Table 2.4). The only exception was within test 1; at this point all the pigs were naïve to the test and there was very little correlation at all. There was significant agreement between tests and levels of consistency in rank order aggressiveness between sexes were similar, although males were slightly more consistent between days 60 and 95 and days 60 and 130.

Table 2.4 Correlation coefficients of attack latency residuals within test (between test pairs) and between tests, with levels of significance indicated. Residual correlation (top), raw data coefficients (middle) and sample size indicated in brackets for each analysis

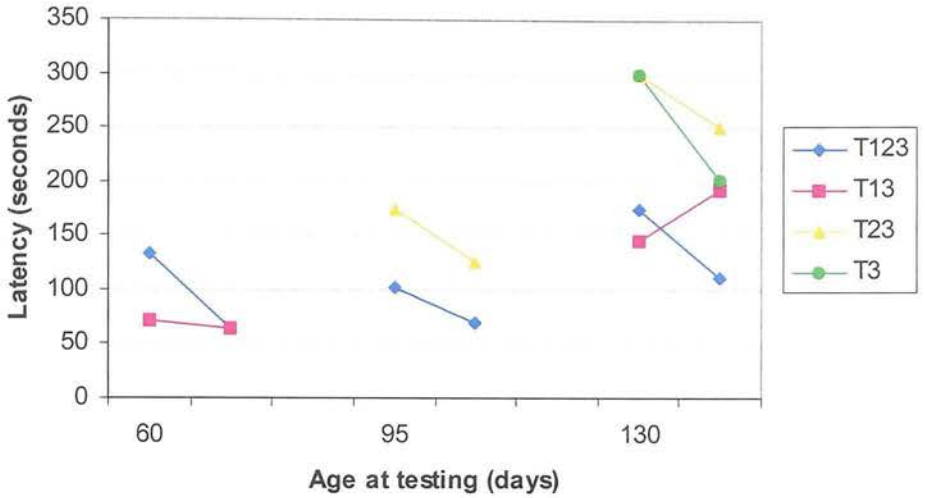
	Within Test			Between Tests		
Sex	1	2	3	1 & 2	2 & 3	1 & 3
Female and Male (163)	0.107 0.305*** (112)	**0.433***** 0.558*** (114)	**0.393***** 0.533*** (163)	**0.301**** 0.314** (90)	**0.427***** 0.529*** (114)	**0.277**** 0.237* (112)
Female (85)	0.177 0.265* (64)	**0.470***** 0.554*** (62)	**0.358***** 0.427*** (85)	**0.288*** 0.266* (53)	**0.431***** 0.507*** (62)	**0.262*** 0.249* (64)
Male (78)	-0.004 0.365* (48)	**0.375**** 0.538*** (52)	**0.475***** 0.494*** (78)	**0.330*** 0.366* (37)	**0.402***** 0.474*** (52)	**0.305*** 0.195 (48)

2.3.2 Treatment effects

2.3.2.1 Effects of experience

Within each treatment pigs took longer to attack on their first testing experience (a) than in the subsequent test (b) (Figure 2.3). This was significant in T123 (Figure 2.4, 123, 1a & 1b, $T^+ = 85.0$ $p < 0.001$) but only a trend in T23 (2a & 2b, $T^+ = 46.0$ $p = 0.080$) and T3 (3a & 3b, $T^+ = 40.0$, $p = 0.078$) and did not reach significance in T13. This was also true when median latencies for males and females were examined separately (Table 2.1), although the differences were no longer significant, except for the females of treatment 123 ($T^+ = 249.5$, $p < 0.001$).

Pigs with greater experience appeared to have faster median attack times in the last test (two previous experiences, T123 = 354.0 s; one previous experience, T13 & T23 = 374.0 s; no previous experience, T3 = 393.0 s), but this difference was not significant. Females with prior test experience on day 95 tended to be faster to attack than those without ($U = 149.5$, $N = 62$, $p = 0.075$). Males did not differ in latency. There was generally little evidence for an effect of experience on absolute aggressiveness beyond the first test in females or males.



	Range (seconds)					
	Day 60		Day 95		Day 130	
Treatment	1a	1b	2a	2b	3a	3b
T123	47 - 300	28 - 247	28 - 300	26 - 300	48 - 300	34 - 300
T13	49 - 254	25 - 253			67 - 300	67 - 300
T23			52.5 - 300	50.5 - 245	108 - 300	79.5 - 300
T3					72 - 100	20.8 - 300

Figure 2.4 Attack latency by treatment group at paired tests 1a and 1b (age 60 days), 2a and 2b (age 95 days) and 3a and 3b (age 135 days), with table detailing the interquartile range

Examining the occurrence of mounting on day 130, pigs with more testing experience were less likely to mount (percentage mounting, 3a, T123 = 21 %; T23 + T13 = 71 %; T3 = 28 %; $\chi_2 = 5.47$, $p = 0.065$; 3b, T123 = 14 %; T23 + T13 = 30 %; T3 = 28 %; $\chi_2 = 5.01$, $p = 0.081$). Comparison of test outcomes (attacking, not attacking and mounting) found that treatments with greater experience (T123) were more likely to attack and less likely to mount and the opposite was true of pigs with less experience (Table 2.5).

Table 2.5 Test outcome (attack, mount, or time-out) on day 130, percentage of pigs (and actual numbers = N) in each treatment

Test 3 outcome	Treatment							
	123		13		23		3	
	%	N	%	N	%	N	%	N
Attack	**61**	55*	**64**	14	**38**	9*	**44**	12
Mount	**14**	13	**27**	6	**25**	6	**44**	12*
Attack/ Mount	**13**	12	**5**	1	**21**	5*	**7**	2
Timed-out	**11**	10	**5**	1	**17**	4*	**4**	1*

Chi analysis with all treatments (as table above), $\chi_9 = 17.42$, $p = 0.042$, cells with greatest contribution to chi statistic indicated*

When comparing the latency data using Spearman's Rank Order correlation it should be noted that the level of significance increases with sample size, so comparison of the p values obtained using Spearman's between treatment 123 (N = 90) and the other, smaller, treatment groups should made with this proviso. Within test point 3 (comparison of a & b, on day 130), rank order consistency (relative latency to attack) generally improved with experience (Table 2.6). Pigs with two previous experiences at the last testing point showed the highest correlation (T123, $r_{88} = 0.456$, $p < 0.001$), followed by those with no previous experience (T3, $r_{25} = 0.388$, $p = 0.046$) and those with one previous experience (T23 & T13, $r_{44} = 0.283$, $p = 0.057$).

Increased experience improved consistency between test points (comparison of test point 1, 2 and 3). Treatment 123 showed a greater (and significant) correlation in rank order aggression between the first and last tests (Table 2.6) compared T13, without the intervening test. An additional testing experience on day 60 did not improve rank order consistency in T123 compared to T23, but there was greater consistency in the occurrence of attacks (T123, $p < 0.0001$; T23, not significant) (Table 2.7). Thus, greater testing experience increased consistency in relative rank order aggressiveness over time, and improved consistency in responses (attack or not) within test (a & b) and between adjacent tests (1, 2, 3).

Table 2.6 Rank order consistency (Spearman's correlation coefficients) of attack latencies, by treatment, within test (between test pairs) and between tests. Coefficients shown are for residuals (top) and raw data (bottom), with significant coefficients (and levels) indicated

			Within Test			Between Tests		
Sex	Treatment	N	1	2	3	1 & 2	2 & 3	1 & 3
Female and Male	123	90	**0.223*** **0.341****	**0.381***** **0.526*****	**0.456***** **0.563*****	**0.301**** **0.314****	**0.271**** **0.540*****	**0.420***** **0.220***
	13	22	-0.334 0.223	-	**0.536**** **0.697*****	-	-	0.342 0.365
	23	34	-	**0.692***** **0.755*****	0.072 0.315	-	**0.467*** **0.472***	-
	3	27	-	-	**0.388*** **0.439***	-	-	-
Female	123	53	0.177 **0.283***	**0.453***** **0.506*****	**0.463***** **0.501*****	**0.288*** **0.266***	**0.435***** **0.499*****	**0.261** [a] 0.216
	13	11	-0.327 0.281	-	**0.555** [c] 0.529	-	-	0.236 0.409
	23	9	-	**0.733*** **0.823****	-0.500 0.398	-	0.400 0.477	-
	3	12	-	-	0.364 0.015	-	-	-
Male	123	37	-0.004 **0.399***	0.157 **0.507*****	**0.451**** **0.494****	**0.330*** **0.366***	**0.376*** **0.464****	0.274 0.217
	13	11	-0.445 0.096	-	**0.745**** **0.976*****	-	-	0.436 0.190
	23	15	-	**0.686**** **0.693****	**0.607*** 0.241	-	**0.475** [b] **0.537***	-
	3	15	-	-	0.275 **0.609***	-	-	-

Trends nearing significance [a] = 0.059; [b] = 0.074; [c] = 0.077

Table 2.7 Degree of association in the occurrence of attacking within test (Fishers exact probability values). Shading indicates points when the different treatments had received the same number of test experiences, i.e. naïve pigs (palest grey), pigs with one previous experience (mid-grey) and those with experience at two previous time points (T123 only)

	Treatment			
Test Day	123	13	23	3
60 (1a&b)	0.002	NS	-	-
95 (2a&b)	0.0002	-	0.009	-
130 (3a&b)	0.0001	0.002	0.099	NS

Examining the sexes separately, males were generally consistent in their relative latency to attack within test 3 (T123, $r_{35} = 0.451$, $p < 0.005$, T13 & T23, $r_{24} = 0.610$, $p < 0.001$), with the exception of those with no prior testing experience (T3). Females from T123 were moderately consistent within the last test ($r_{51} = 0.463$, $p < 0.001$). The coefficients for T13 and T3 were of similar magnitude (albeit not as high as in the male pigs) but were not significant (Table 2.6).

2.3.2.2 Effects of age

As reported earlier, overall latency to attack increased by day 130. This was also evident when comparing naïve pigs at the different points (summed medians, naïve pigs day 60 = 282.5s; day 95 = 259.0s; day 130 = 393.0s, $H_2 = 4.903$, $p = 0.086$). The largest difference was between pigs tested for the first time at days 60 and 130 ($U = 1096.5$, $p = 0.026$). Comparing the latency to attack in the last test, early-first-testing-experience pigs (T123 & T13) were faster to attack than late-first-testing-experience pigs (T23 & 3) (test 3a, median latency, early = 166.5s, late = 300.0s, $U = 2319.0$, $p = 0.044$; test 3 summed, early = 350.0s, late = 430.0s, $U = 2357.0$, $p = 0.07$). The early experience group did contain the pigs with the greatest overall testing experience (T123), however, as detailed in the previous section, these pigs were not significantly faster to attack than the other treatments in the last test.

Early first experience pigs (day 60) attacked more than late experience (day 95+) pigs on day 130 (test 3a, 60 % early experience attacked, 43 % late experience attacked, $\chi_1 = 3.93$, $p = 0.047$). When analysed separately this trend only remained true of the females (78 % early,

57 % late, $\chi_1 = 3.330$, $p = 0.068$), although the percentage of tests ending in an attack suggests a similar, non-significant, effect in male pigs (test 3, early 42 %, late 35 % attacks). The occurrence of mounting in the last test was greater in late experience pigs (proportion mounting at least once, early experience 29 %, late 49 %, $\chi_1 = 6.443$, $p = 0.011$).

Early experience pigs were more consistent in rank order aggression within test on day 130 than late experience pigs (early, $r_{110} = 0.460$, $p < 0.001$; late $r_{49} = 0.253$, $p = 0.073$). Early experience pigs were also more consistent in the occurrence of attacking or not-attacking (early, $\chi_1 = 725.96$, $p < 0.001$; late $\chi_1 = 9.56$, $p = 0.002$).

2.3.2.3 Recent versus distant experience versus age at first testing

As detailed in previous sections consistency within tests (between test pairs on consecutive days) was greater than consistency between tests that were more than 30 days apart, thus length of time between testing would be expected to influence consistency. Pigs in T23 and T13 had the same number of previous test experiences at test 3, but groups 23 had a more recent previous experience of the test. The groups also differed in the age at first testing, with 13 having an earlier first testing experience. Previous analysis suggested that earlier first testing experience should improve consistency, which contradicts the hypothesis that recent experience should improve consistency. Comparing T13 and T23 more recent experience did not appear to improve consistency, as pigs that had a longer period between tests (but experiencing the test when younger) showed greater consistency in attacking within test 3 (T13, $p = 0.001$, T23, $p = 0.099$). Pigs with more distant (earlier) experience were more consistent in rank order aggressiveness within test 3 than those tested more recently (test 3, T13, $r = 0.536$, $p < 0.01$, T23, $r = 0.072$, not significant). The more distant experience group showed a significant association in attacking or not attacking between days 60 and 130 when looking at just the first test of the test pairs (1a & 3a, $p < 0.05$). Between tests however, the more recent experience group showed greater rank order consistency in latency to attack (T23, days 95 and 130, $r_{22} = 0.467$, $p = 0.021$) than the treatment with tests further apart (T13, 60 and 130, $r_{20} = 0.342$, not significant). The only difference between the sexes was that the females, but not the males, with more distant (earlier) testing experience were faster to attack on day 130 (test 3a, median latencies T13 = 77.0s, T23 = 295.0s $U = 21.0$ $p < 0.05$).

2.4 Discussion

2.4.1 Changes in speed of attacking, relative aggressiveness and consistency to attack over time

Male pigs took longer to attack as they got older, at least in part due to an increase in mounting behaviour. This was true even for pigs naïve to the test situation. The latency to attack in females did not change after the first (novel) experience, which has also been reported in other studies on female pigs (Janczak et al., 2003).

Pigs were consistent in attacking or not attacking, with many pigs attacking in every test and a small number that never attacked. Relative 'rank-order' aggressiveness was also consistent over time. The largest change in consistency was between tests 1a and 1b, possibly due to the novelty of test 1a to naïve pigs, although this effect was less pronounced in older naïve pigs at first testing. Rank order latency of attacking was consistent between adjacent tests (days 60 & 95, 95 & 130), but not between days 60 and 130, suggesting that although there was generally consistency over time, there were some changes in relative rank order between the start and end of the study. Both sexes were consistent, although this may have occurred for difference reasons. Females were likely to be consistent in attacking, whereas several males were very consistent mounters and therefore more likely to be consistent non-attackers. The consistency found overall, and in the female pigs, compares well with other studies, for example, consistency in aggressiveness was found over a period of 16 weeks in female pigs (Janczak et al., 2003) and over 9 weeks in mixed sex pigs (D'Eath, 2004), using social confrontation tests.

There were a greater number of pigs that consistently attacked compared to pigs that never attacked. Aggressiveness in the RIT may either be more consistent than being non-aggressive, or alternatively, being not very aggressive or 'less aggressive' (as opposed to never or non-aggressive) might be a better definition of the opposite end of the trait spectrum. In breeding experiments on mice of diverging aggressiveness, selection for long attack latency has generally been less effective than selecting for short attack latency (van Oortmerssen & Bakker, 1981). One explanation for this being that, "*a minimum amount of aggression in the animals is protected by the genotype*", as it would not be sensible from an adaptive perspective for an animal never to be aggressive (van Oortmerssen & Bakker,

1981). The generally high attack rate meant that most pigs attacked at least once, suggesting that perhaps there is a basal level of attacking, even in non-aggressive pigs.

2.4.2 Sex effects

The sex of the pigs being tested is an important factor to consider, as males and females clearly behaved differently from day 95. Males took longer to attack, either because they were less aggressive, or because sexual motivation masked any underlying aggressiveness. The exact mechanism is unclear. Mounting was more common in males. Even though the pigs were nearing maturity by the test on day 130, mounting had been consistent from a very young age (from day 60), prior to sexual maturity (usually 5 to 8 months (Hughes & Varley, 1980)). Nor is it clear whether mounting was just a problem encountered on this occasion, as it is rarely recorded or reported in the literature. The occurrence of attacks (71 %, 68 % and 57 %) is comparable to D'Eath & Burn (2002) (55 % and 67 %) and Erhard & Mendl (1997) (63% and 74%), although the latter did define an attack as a minimum of only one bite rather than a series of bites. Erhard & Mendl (1997) reported a mounting rate of 1 % on day 1, increasing to 6 % on day 2. No difference between sexes was found and there was no mention of whether it was just the males that mounted. Interestingly latency did decrease between 7 and 11 weeks, when both attackers and non-attackers were included in the analysis, but not when attackers-only were analysed. Mounting was not mentioned by D'Eath (2004), although the females were quicker and more likely to attack. Fraser (1974) found dominant pigs were more likely to mount an unfamiliar pig, and that male pigs tended to be dominant over females (Gonyou, 2001). It could potentially have been the dominant (predominantly male) pigs that mounted, but numbers are too small to say decisively. Comparison with other measures of dominance might be able to confirm a link (to be discussed in Chapter 3). Another factor that deserves further investigation is the possible link between aggressive behaviour in the mix and mounting; again it was male pigs that showed more aggression in the first half of the mix.

Links between sexual behaviour and aggression have been made in other species and the occurrence of mounting in some males from a young age may have been due to differences in sexual maturity, with some males maturing sooner than others. Testosterone in male mice has a controlling function that reduces or eliminates attacks from male residents on lactating female intruders (Whalen & Johnson, 1987). Additionally, the presence of novel females may have been stimulating to the male residents. In male guinea pigs sexual behaviour

directed to females in the home group is suppressed, whereas it is stimulated by the presence of unfamiliar females, possibly to reduce inbreeding (Hennessy et al., 2003; Cohn et al., 2004). There are no data on the frequency of mounting behaviour in the home groups post weaning and so it is not possible to determine whether the test situation itself was stimulating the males to mount.

In addition to males attacking less, females were more likely to attack male intruders on day 130, perhaps in response to male intruders trying to mount the residents. A similar effect has been noted in mice, except that females rejecting male mount attempts led to male attacks (Blanchard et al., 1984). It could be that the difference between the sexes seen here is an adaptive behaviour. Female pigs in natural conditions would not associate with unfamiliar females or males (apart from when mating) and juvenile male pigs leave the family group at around 7-8 months old (Gonyou, 2001), which may be precipitated by increased aggression from the females of the group. Males, on the other hand, might be more receptive to unfamiliar females as they may be potential mates. It can be concluded that there appears to be an underlying tendency for differences between the sexes in attacking, which may become more apparent in mature pigs. These differences require further investigation to understand why they were so evident in this study and yet have not reported to this extent previously.

2.4.3 Effect of increased testing experience

The results suggest that experience improved the consistency of response, both within and across tests, but did not affect the speed at which the pigs attacked. There was a difference between the sexes: after the initial 'novel' experience, females did not change their latency to attack with greater experience, whereas more experienced male pigs were faster to attack in the last test than those with less experience. Examining the median latencies (Table 2.1), the experienced males (T123) took longer to attack on day 130 than they had done on days 60 and 95, although the increase was not as great as those with less experience. The difference between groups was probably a result of experienced pigs being less likely to mount.

There are several examples of repeated exposure to unfamiliar animals reducing the frequency, duration or speed of subsequent attacks (Parmigiani & Brain, 1983; van Putten & Bure, 1997; Giersing & Andersson, 1998; Gariepy et al., 2001). In these tests the interaction is generally longer than an initial attack and subjects gain experience of fighting and

probably winning or losing a contest. Other than a priming effect within test pairs, repeated RIT (where animals only experienced the initial attack) did not cause pigs to attack an unfamiliar animal more often or more rapidly. D'Eath (2004) found a small, but significant, decrease in latency to attack with repeated tests using the same procedure. The reasons for the disparity between this study and that by D'Eath (2004) are likely to be due to differences in ages or experience of the pigs. In the D'Eath (2004) study, the pigs were far more socially experienced and this repeated mixing experience may have resulted in the pigs being more likely to approach and attack an unfamiliar pig. The time period between confrontation experiences was also much less, so that by the second pair of RIT at day 80, the pigs had already experienced two mixes and a pair of RIT, within a period of 35 days. In addition, if greater experience increases consistency between tests then less variation within animals would be expected. In support of this, decreases in median latency of just 15 and 7 seconds (N=112) were sufficient to be significant in the experiment of D'Eath (2004), whereas a decrease of similar magnitude (21.5 second difference between tests 1 and 2, N = 90) in this study was not. There may also have been an effect of age. The oldest pigs tested in the experiment of D'Eath (2004) were approximately 113 days old and a small (non-significant) decrease was found between the ages 60 and 95 days old here, but not after 130 days.

2.4.4 Effect of age at first test

Early first experience pigs were more likely to attack, were faster to attack and more consistent than late first experience pigs. In addition, comparison of recent versus distant experience confirmed the finding that early exposure to the test is important. Greater consistency in attacking would be expected between recent experiences, whereas the opposite was found in this case, with pigs having a more distant (early) prior experience being more consistent than those with recent (late) experience. The type and quality of early-life social experiences has been demonstrated to be important in behavioural, physiological, immunological and neural development in pigs (Worsaae & Schmidt, 1980; Worobec et al., 1999; Olsson et al., 1999; Kanitz et al., 2004; Poletto et al., 2006) and other species (Potegal & Einon, 1989; Veissier, 1994; Kaiser et al., 2003). The impact of these early life events can often last throughout lifetime. For example, pigs reared in barren environments pre-weaning have been shown to be more aggressive when adults, than those reared in enriched conditions (de Jonge et al., 1996). Group-reared subordinate rats are not aggressive towards intruders, whereas isolation-reared rats are, and yet subordinate animals removed from colonies when approaching maturity and subsequently housed in isolation for over 5 months

will continue to show subordinate-typical responses to intruders, rather than isolation-reared responses (Lore & Stipo-Flaherty, 1984). The findings of this study concur with the evidence for the impact of social experiences in young animals, demonstrating that earlier experience of the RIT has more profound effect on aggressiveness in subsequent tests, than later experiences of the same test.

2.4.5 Inter-test consistency, RIT aggression versus mix aggression

There was no correlation between pigs that were immediately aggressive in the mix and the fastest attacking pigs in the RIT. As both the RIT and mixing were both tests of social confrontation, some similarities in the response of individuals was expected. However, the tests were very different. The RIT is designed to provoke aggression, with the resident having the advantage of size and territory, and with little space for either pig to avoid the other. In contrast, pigs were introduced to a novel pen during mixing, and so some of the initial period post mixing is likely to have been spent investigating their new surroundings. The RIT is a measure of relative aggressiveness alone, whereas aggression measures from mixing will be altered by the animal's assessment of it's own fighting ability, the weight of the other pigs present (Rushen, 1988) and how aggressive opponents are (Erhard et al., 1997). D'Eath (2002) did find differences in involvement in fighting during mixing, between RIT aggressive and non-aggressive pigs, but this was only evident the day after mixing. This illustrates the importance of looking at the different nature of aggression measures. It may be that during mixing, aggressive animals can be characterised by differing agonistic tactics, such as bullying (D'Eath, 2002), as opposed to being involved in more outright fighting. A measure consisting of all aggressive acts performed in the first 30 minutes of the mix may, therefore, have been too simplified to pick up any differences. This will be examined in more detail in Chapters 3 and 4. Alternatively, the RIT methodology may not be functionally comparable to aggressiveness displayed during mixing.

2.4.6 Summary

There was evidence of consistency over time and definable differences between individuals consistent with aggression being a trait. Females were consistent over time in absolute aggression (latency to attack), rank order of aggressiveness (relative latency) and the occurrence of attacks, so it was possible to define females as aggressive from a young age. However, responses can be over-ridden or altered by other influences common to certain age

or sex groups. The effect of these influences and resulting behaviour expressed will be moderated by factors particular to the individual, including its temperament. When using tests of social interactions the influence of over-riding factors should be examined, particularly when looking for stability in repeated tests over long periods of time, where situational factors (such as sexual motivation) may alter. In the case of the Resident Intruder Test, it did not appear to be a reliable test of aggression in male pigs and may in fact be misleading, as a consistent non-attack response by males may be more indicative of maturation, or sexual motivation, than aggression. This study did demonstrate that even well before sexual maturity the effects of mounting should not be dismissed. Aggressiveness in the RIT was not directly comparable to a measure of initial mixing aggressiveness.

CHAPTER 3

BEHAVIOURAL STRATEGIES DURING MIXING AT WEANING IN MALE AND FEMALE PIGS OF DIFFERING AGGRESSIVENESS; EFFECTS ON FIGHTING SUCCESS, INJURIES RECEIVED, DOMINANCE STATUS AND CORTISOL

3.1 Introduction

Commercially reared piglets are usually mixed at weaning, typically at around one month of age. This process involves removing the sows and then combining piglets from several litters together, to create suitably sized groups for management purposes. Merging unfamiliar litters usually leads to considerable fighting and aggression between pigs (e.g. Pitts et al., 2000; Bolhuis et al., 2005; D'Eath, 2005); this is detrimental to welfare, as it results in many pigs receiving wounds (referred to as skin lesions) (e.g. Turner et al., 2006) and in extreme cases may even lead to death (McGlone et al., 1981). Mixed pigs also show physiological responses indicative of high levels of stress (Otten et al., 1999; Merlot et al., 2004), which in turn may have detrimental effects on reproduction (Madej et al., 2005), immunity (de Groot et al., 2001) and production parameters such as growth (Wellock et al., 2003) and meat quality (Terlouw et al., 2005). In contrast to the aggression observed when unfamiliar pigs meet under commercial conditions, aggression is rare when piglets are first introduced into social groups in wild or free-living domestic pigs (Newberry & Wood-Gush, 1986; Petersen et al., 1989). Social integration begins when piglets are between 7 and 14 days old. At this point the litter abandons the farrowing nest and returns with the sow to her original social group, where piglets will encounter unfamiliar pigs of various ages. The most frequently observed social exchange between free-ranging unfamiliar piglets is short-non-aggressive nosing interactions. Other commonly observed behaviours include short aggressive interactions, such as pushing and head-knocks, play (scampering) and mounting (Petersen et al., 1989).

Numerous attempts have been made to understand aggression between pigs at mixing and to establish what elements of the commercial system might be predominantly responsible for the elevated levels of aggression, with the ultimate aim of eliminating or reducing aggression (for reviews see: Petherick & Blackshaw, 1987; Arey & Edwards, 1998; Marchant-Forde & Marchant-Forde, 2005). Methods have included the manipulation of environmental factors, such as the provision of hides and barriers (McGlone & Curtis, 1985), the inclusion of straw (Arey & Franklin, 1995), or the use of chemical interventions (Barnett et al., 1993) and masking odours (Barnett et al., 1993). Others attempts have used physical attributes such as mixing pigs of unequal weight (Andersen et al., 2000), or creating same-sex groups (Colson et al., 2006). Some experiments have tried to imitate the natural system in some way, by for example, allowing piglets of different litters to interact freely before weaning (Parratt et al., 2006). Another approach has been to classify pigs according to individual differences in

responses to the same situation, including differences in aggressiveness. These behavioural differences have also been linked to other social and non-social behaviours and physiological characteristics, including responsiveness to stress (Geverink et al., 2002; Ruis et al., 2002). The purpose of studying these individual characteristics or traits is to enable a better understanding of overall behavioural style or personality and by doing this, minimising aggression through, for example, artificially selecting desirable traits, or altering the composition of mix-groups using specific combinations of individuals (e.g. Erhard et al., 1997).

Comprehensive research into mixing aggression and fighting sequences indicate that different patterns or types of aggression are indicative of different kinds of pigs (e.g. defensive and offensive aggression) (Rushen & Pajor, 1987). Despite this, aggressive behaviours are often merged into one 'aggression' category, which may mask important differences between individuals. Studies often focus purely on aggression at mixing (e.g. Erhard et al., 1997; Giersing & Andersson, 1998; Drickamer et al., 1999). This ignores an important group of individuals, those pigs that do not respond aggressively, yet in free-ranging conditions most pigs would exhibit low/no-aggression. Another sometimes-neglected variable at mixing is whether the type of aggression differs according to characteristics of the actor and recipient, such as familiarity or sex. The importance of sex was demonstrated in Chapter 2, where females were shown to be more aggressive in the resident intruder test (RIT), which is thought to be predictive of mixing aggression (Erhard et al., 1997). The results of Chapter 2 also alluded to a link between individual aggressiveness and mounting, although this behaviour has also been linked with play (Dobao, 1984/85).

The aims of this study were to investigate whether: (i) differences between pigs in the performance of aggressive and non-aggressive behaviour during mixing at weaning can be used to describe distinct mixing strategies; and (ii) if such strategies exist, do they result in differing success in aggressive encounters at mixing and ultimately, differences in social rank? Individual behavioural differences were measured using a detailed ethogram to analyse the behaviour of all the pigs in the mix, rather than a sample of selected animals. This was to gain a greater understanding of individual differences and avoid excluding groups of pigs that may otherwise be ignored or engulfed by less discriminating categories. If aggression functions to create a dominance hierarchy, then parallels between dominance score and success, or involvement in fights, would be expected. Social rank was assessed using dominance in a group-feeding test as pigs approached slaughter weight and a score of

contests won in relation to total contest involvement was used as a measure of success during mixing. Body weight, which has been linked with fighting success and dominance (Drickamer et al., 1999; O'Connell et al., 2003), was also recorded and salivary cortisol levels pre and post mixing were used as an indicator of stress reactivity.

3.2 Methods

3.2.1 Animals and housing

The subjects were the progeny of 19 first parity sows, artificially inseminated with dam-line semen (two boars). The pigs underwent the same husbandry practices and occupied the same buildings as non-experimental farm animals at all stages. Artificial light was used in all buildings between 0730 and 1600 hours, although some natural light was also present. All pens were cleaned daily and straw was provided in all growing accommodation. Temperature and ventilation were controlled by fans (farrowing house) or automatically adjusting ventilation flaps. The sows and their litters were kept in standard concrete floored farrowing crates (pen 1.50 x 3.14 m), with a slatted dunging area at the rear and a heated piglet kennel at the sow-head-end (0.54 x 2.22 x height 1.05 m, temperature 30 °C). The crate floor was covered with wood-shavings and some straw. Litters were born over a six-month period. Piglets were identity tagged within 24 hours of birth with these piglet-tags replaced at weaning. It was normal farm practice not to clip teeth or dock tails. Of the 225 piglets born (mean 11.8 ± 3.5 SD piglets per litter), 218 were born alive and 177 survived to weaning (mean 8.6 ± 1.6 SD per litter). A further 14 pigs died between weaning and slaughter. Mortality post-weaning was in most cases due to infection with PMWS (Post-weaning Multi-systemic Wasting Syndrome). When the litter reached 18 days old approximately 0.02 kg creep-feed was scattered on the crate floor each morning (Scotlean Silver Pellets, ABN), to introduce the piglets to pelleted feed.

At weaning 12 wean-mix-groups were formed by mixing together two litters of similar age and size (mean group size 16.7 ± 3.9 SD). Weaning took place when the younger of the two litters reached a minimum age of 27 days (mean age 33.7 days ± 3.4 SD; mean weight 9.95 kg ± 1.16 SD). Weaning pens consisted of an unheated kennel (1.25 m x 1.75 m) with some straw and an open concrete floor area (2.05 m x 1.75 m) with a slight slope to the dunging area. The dunging area contained two drinkers and the kennelled area contained a four-space feed hopper. Piglets were fed the same pelleted feed that they had been offered in the farrowing crate. On two occasions there was a disparity in the size of the litters to be mixed. To account for this the large litter was split in half, with each half mixed into separate wean-groups. On three occasions there were no experimental (dam-line) litters available of the correct age and size; so litters were

paired with non-experimental farm litters (terminal sire). The farm litters had the same experience and were the same age, but were born to multiparous sows.

When the smallest pigs in each group reached 20 kg the group was moved to grower accommodation and at 40 kg, to finisher accommodation. Grower and finisher pens were of the same design, with a straw-bedded area (grower, 1.8 x 5.0 m, finisher, 2.35 x 6.00 m) containing a feed hopper (feedface length 0.9 m, growers four-spaces, finishers three-spaces) and a dunging passage (grower, 1.8 x 1.8m, finisher 2.35 x 2.35 m) with two drinkers. Pigs were fed a standard commercial dry pelleted feed (Scotlean Silver Pellets, Scotlean Prime Link, LC Rearer and Finisher Pellets ABN).

3.2.2 Procedures and measures

3.2.2.1 Pre-weaning

Body weights were recorded on days 1, 7, 14, 21 and 28 from birth. Day 1 weights were recorded at 0800 or 1600 hours, depending on birth time, so that piglets were between 8 hours and 24 hours old. Weights on all other days were recorded at 0800 hours. 'Body dimensions' of length (crown to rump), height at shoulders and girth of chest (just behind forelimbs) were recorded on days 1, 7, 14 and 28. Ano-genital distance was measured on day 1.

3.2.2.2 Weaning mix

On the morning of weaning the piglets were numbered using permanent marker and given a litter-specific mark with coloured stock marker. The position and number of any fresh skin lesions were noted. Litters were then moved, youngest first, from the farrowing house to weaning pens. Behaviour was recorded for five hours (colour digital camera, wide-angle lens) on the day of mixing and the day following mixing. The camera was positioned so that all areas of the pen were visible, including inside the kennelled area. After recording had finished on the second day, the numbers and position of fresh lesions were recorded. Lesions were defined as raised red marks (resulting from bites). The position was noted as either on the left or right side of the body and further sub-divided by ears, front (area forward of shoulder blades including front limbs and head), mid/flank (torso between front and hind limbs), or rear (hind limbs and tail). The number and position of severe lesions were also noted; these were defined as bleeding lesions. The video recordings were analysed using The Observer (v. 5.0.31, Noldus Information

Technology, The Netherlands). The actor and recipient of all ethogram behaviours were noted (Table 3.1) for the first 30 minutes (up to the saliva samples being taken) and for a further two hours, between hours 1 to 2 and 4 to 5 post mixing. The winner of all fight or mutual pushing events was recorded; the winner caused the other to cease fighting/pushing and move/turn away. When pigs ceased to fight without moving away from each other and then subsequently resumed fighting again a short time after then this was classed as the same fight (recorded as fight pause). When both pigs appeared to cease fighting at the same time and the winner was not clear the fight was recorded as undecided.

3.2.2.3 Salivary cortisol analysis

Saliva samples were taken for cortisol analysis. The pigs voluntarily chewed the end of a large cotton bud. The cotton end was placed in a salivette and spun in a centrifuge at 3000 rpm for five minutes. The saliva was then decanted into cuvettes, frozen at –28 degrees centigrade and stored until analysis. Sampling was performed in the morning (between 0900 and 1100 hours). Samples were taken 24 hours before mixing, 30 minutes, 24 hours and 7 days after mixing. Analysis of the samples was performed by standard radioimmunoassay (COAT-A-COUNT® cortisol kit, TKC05, Diagnostic Products Corporation, UK) (Tunn et al., 1992). Samples at all time points were analysed for 94 pigs. Due to the large number of samples, duplicates and standards, three separate analyses had to be performed. The detection limit was 0.475 ng ml^{-1} and medium intra-assay coefficients of variation were 12.0 %, 7.8 % and 11.1 %.

Table 3.1 Ethogram of behaviours recorded during the weaning mix

Behaviour Class	Measurement	Behaviour	Definition
Aggression	Duration and Frequency	**Fight**	Two pigs simultaneously directing bites/head-knocks to each other in quick succession
		Attack (Front or Body)	One pig directing a series of bites or head-knocks to the front (head and shoulders) or body of recipient.
		Fight pause	Brief cessation of fighting behaviour with pigs still in contact.
		Push	Leaning bodies together, may circle or move forwards whilst in contact.
	Frequency	**Chase**	One pig pursues another, pursued pig flees.
		Bite	Brief open-mouthed assault to any part of the body of another pig.
		Interrupt Fight	A third pig intervenes between attacking pigs, resulting in the fight resuming as before, the fight stopping, or a new fight starting between one of the attacking pigs and the intervening pig
		Short Aggressive Interaction (SAI)	A brief assault, not biting, includes single occurrences of head-knocks and shoves.
Social	Frequency	**Short Non-aggressive Interaction (SNAI)**	A brief contact with the nose, usually to the nose or face of the recipient.
		Displace	Moves another pig from a resource (bedding, feeder and drinker).
		Avoid	Orients body away from another pig, without the avoided pig making physical contact.
		Mounting	One pig rests ventral surface of body on dorsal surface of another, actors front limbs raised off the ground.
		Lie next to	Rests with ventral or lateral surface in contact with the ground within 0.5 metres of another pig.
Other	Duration and Frequency	**Rubbing/ Rolling**	Scratching motion where contact is made between the body and walls, pen fixtures or floor.
	Frequency	**Scamper**	Playful running motion.
		Straw Play	Taking straw in the mouth and shaking head or throwing straw in the air using the nose.
Responses (to attacks, pushing and mounting)	Frequency	**Turn Away/Yield Push**	During mutual fighting or pushing one pig turns its head and/or body away from the other pig moving out of mutual contact.
		Flee	Runs away.
		No Response	Stands still and appears to ignore actor.

3.2.2.4 Resident intruder test

The methods for this test have been described in detail in Chapter 2. In summary, a section of the home pen of the experimental animal (resident) was sectioned off and an intruder of 72.7 % (± 5.1 SD) the weight of the resident pig was introduced. The test was terminated when an attack occurred (rapid and persistent biting), or five minutes after the resident made contact if there was no attack. Following termination of the test the pigs were separated immediately. At each testing point the 'resident' pig was tested twice, once on each of two consecutive days, with a different intruder on each occasion. Occurrence of attacks and latency to attack were recorded. Pigs were tested aged 60 (RIT 1), 95 (RIT 2) and 130 (RIT 3) days.

3.2.2.5 Food competition test for dominance rank

A group feeding competition test was used to determine dominance rank. Because of the large group sizes, it was decided to adapt the standard food competition test method. Usually one quantity of food is placed in a pen and observations continue until all of the feed has been consumed, at which point the test ends. However, it was thought that this would not provide enough information on the status of the majority of pigs in the group, as the feed would be monopolised by the most dominant subset of the group. Therefore the test continued after the initial quantity of food had been consumed, with the most dominant animals removed from the pen. Pigs were food deprived to ensure that they were motivated to feed in the test. The pigs were moved into a pen without a feeder as soon as the lights were put on (0800 hours), preventing the morning peak in feeding activity. The test was performed seven hours later, at 1500 hours. The pigs were numbered and an area of approximately 0.5 m^2 in the centre of the pen cleared of debris. One scoop of feed (approximately 1 kg) was placed in the centre of the cleared area. The identity of the actor and recipient of 'attacks' (bites, knocks, pushing) and 'avoids' (actor turned away from recipient without being attacked) was recorded. Once the feed had been consumed the two animals that had initiated the most attacks were removed from the pen. If it was not possible to clearly distinguish two dominant animals then only one was removed. The procedure was repeated with the amount of feed reduced to compensate for the pigs that had been removed. The order of removal from the group was used to assign rank-scores to the pigs (i.e., first removal pigs were ranked 1, then 2 and so on). The test continued until either there were only two pigs left, or where after two repeated tests no pig was distinguishable as dominant. The latter occurred on six occasions, with between three and six pigs left in the test arena with indistinguishable ranking.

3.2.3 Statistical analyses

Data were tested for normality and transformed (square-root) where necessary. The observation period (2.5 hours) was divided into five time intervals of equal length: from 0 to 30 minutes (interval 1); 60 to 90 minutes (2); 90 to 120 minutes (3); 240 to 270 (4) and 270 to 300 (5) minutes post-mixing. For each pig the latency to first aggressive behaviour (within the five intervals) was calculated. The duration of attacking, fighting, pushing and fight pause were recorded and analysed individually and summed (total aggression duration) and expressed as either total duration or mean bout duration. Aggressive and social behaviours (see Table 3.1) such as, attacking, SNAI and lying next to, were analysed in terms of the familiarity of the actor and recipient; familiar pigs were littermates and unfamiliar pigs came from different litters. Repeated Measures ANOVA was used to examine the effect of sex and time interval on behaviour frequencies and duration. For the remainder of the analyses, the separate interval data for each pig was summed to give a total frequency or duration.

Relationships between behaviours were investigated using a Pearson correlation, or Spearmans rank order correlation coefficient for category and ranked data. Success scores were calculated as fights won minus those lost, subdivided by total fights (including those with undecided outcomes): high success pigs had higher and positive scores. Categories (high/low) were formed for the variables: total aggression and fighting frequencies; received aggression; lesion counts; mounting; dominance rank; body weight, group weight asymmetry, group size and cortisol level, according to whether the individual mean was higher or lower than the overall mean value. For success scores there was also an intermediate category to account for pigs that did not fight (zero scores). The group weight asymmetry between litters mixed at weaning was calculated by subtracting the mean weight of the lighter litter from that of the heavier litter. Differences between the categories were analysed using a t-test or one-way ANOVA. Frequencies in discreet categories were analysed using Chi Square contingency tables. For example, the occurrence of mounting (yes/no) in the resident intruder test compared to high/low frequency of mounting in the mix.

Changes in cortisol between sampling points were analysed using paired t-tests. All pigs were saliva sampled and samples for 94 pigs were analysed. It was sometimes not possible to collect sufficient saliva and so only 90 samples for 30 minutes post mix and 93 samples for day 7 post-mix were successfully analysed.

Principle components factor analysis (PCA) was used to visualise patterns and clustering in the behaviour measures that might indicate associations between them, and also to calculate the amount of variation in the data that could be explained by these associations. Components were created using a varimax rotation and only those with an eigenvalue greater than one were examined. The PCA analysis provided loading values for the measures entered into the analyses for each of the component dimensions and also scores for each pig in relation to these dimensions. The PCA was initially formed using only the mix behavioural data. Behaviours that did not load highly on any axis were removed and those clustering closely together were combined. Other measures (success, cortisol, dominance, weight and lesions) were then fitted to the main PCA. Individual pigs were categorised based on their scores (whether they loaded on the positive or negative aspect of each dimension) and these categories were used to interpret any differences in the measures taken using one-way ANOVA.

3.3 Results

3.3.1 Overall effects across time intervals

Frequency of aggression was greatest during the first interval and declined over time (Table 3.2). Duration of attacking and fighting also decreased initially, but then increased during intervals 4 and 5, although there was an effect of sex. Males displayed more overall aggression, including attacking, fighting, interrupting fights and SAIs and spent a greater amount of time performing aggressive behaviours than females, particularly one-sided attacking. Males also had higher overall success scores than females (success = contests won – lost / won + lost + undecided, Mann-Whitney $U = 4137.0$, $p = 0.029$) and were faster to perform an aggressive behaviour (median latency to first aggressive behaviour, males = 1644.0 s, females = 3878.0 s, $U = 3054.5$, $p < 0.001$). The bout time of fights declined over time in the females, but attack duration remained at similar levels and increased slightly in intervals 4 and 5. In the males there was a small rise in total and bout duration of fighting and a pronounced increase in attacking duration, without a corresponding increase in frequencies (Figure 3.1).

There was an effect of time, but not sex, on chasing, SNAI, received aggression and received mounting, with all except receiving aggression decreasing with time. During the initial mix period males were more likely to mount than females and females were more likely to play than males. The difference between the sexes decreased as the frequency to perform these behaviours declined.

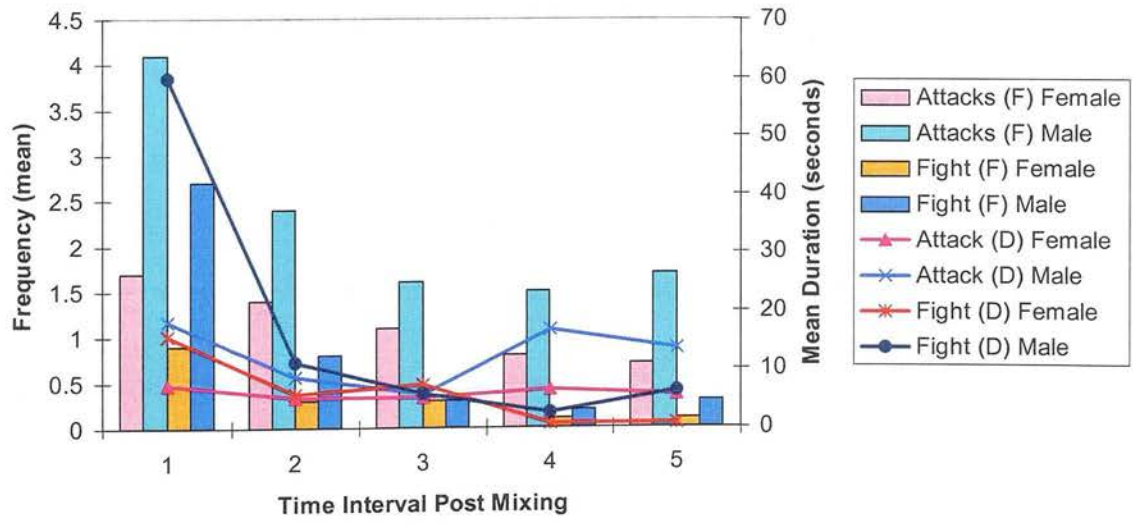


Figure 3.1 Mean frequency (F) and duration (D) of attacks and fights by male and female pigs, during intervals 1 (0 to 30 minutes), 2 (60 to 90 minutes), 3 (90 to 120 minutes), 4 (240 to 270 minutes), and 5 (270 to 300 minutes) post mixing and mean bout duration (± SEM)

Table 3.2 Frequency of behaviours (interval mean) in the weaning mix, by interval and sex, with duration of aggressive behaviours (total mean duration and mean bout duration) and significance level of sex, time intervals and interaction (sex*interval) effects (Repeated Measures ANOVA, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$)

BEHAVIOUR FREQUENCY		TIME INTERVAL MEAN BY SEX 1	2	3	4	5	SEX	TIME	SEX*TIME
Aggression	Female	21.5	11.3	6.5	5.2	4.3	***	***	NS
	Male	28.2	14.8	7.4	7.1	6.9			
Attacks	F	1.7	1.4	1.1	0.8	0.7	***	***	***
	M	4.1	2.4	1.6	1.5	1.7			
Fight	F	0.9	0.3	0.3	0.1	0.1	***	***	***
	M	2.7	0.8	0.3	0.2	0.3			
Interrupt Fight	F	0.2	0.0	0.0	0.0	0.0	**	***	***
	M	0.5	0.1	0.1	0.0	0.0			
Chase	F	0.4	0.3	0.2	0.1	0.0	NS	***	NS
	M	0.5	0.4	0.3	0.1	0.0			
SAI	F	17.9	9.1	4.8	4.2	3.5	*	***	NS
	M	19.7	10.9	5.0	5.2	4.9			
SNAI	F	28.6	18.6	9.9	1.0	1.1	NS	***	NS
	M	27.1	16.4	8.8	1.0	1.2			
Play	F	13.4	6.0	2.4	0.6	0.2	**	***	*
	M	9.2	3.7	1.3	0.3	0.3			
Mount	F	0.9	0.3	0.1	0.1	0.3	***	***	*
	M	2.3	1.7	0.9	0.4	0.4			
Aggression Received	F	11.1	8.8	6.1	11.8	6.1	NS	***	NS
	M	9.9	7.9	7.7	13.0	4.8			
Mount Received	F	1.5	1.1	0.7	0.3	0.3	NS	***	NS
	M	1.7	0.8	0.5	0.3	0.2			
DURATION (SEC)									
Total Aggression	F	23.3	11.1	12.8	7.3	6.5	***	***	***
	M	78.1	20.0	11.8	19.2	19.8			
Attack	F	7.5	5.3	5.3	6.6	5.7	***	***	**
	M	18.3	8.7	5.8	16.7	13.6			
Fight	F	15.7	5.8	7.4	0.7	0.8	***	***	***
	M	59.8	11.3	5.9	2.6	6.2			
Mean Attack Bout	F	1.4	1.0	1.4	1.9	2.2	***	***	***
	M	2.6	1.5	0.9	3.8	4.2			
Mean Fight Bout	F	6.1	2.0	1.9	0.7	0.7	***	***	**
	M	12.6	4.5	1.7	1.9	3.2			

3.3.1.1 Familiar versus unfamiliar opponents

There was a trend for pigs to direct more aggression towards familiar opponents (littermates) overall (Repeated Measures ANOVA, variance ratio$_1$ = 3.25, $p = 0.073$), although unfamiliar pigs received more aggression in the first interval ($vr_4 = 5.55$, $p < 0.001$) (Table 3.3). Males were more likely to be involved in fights ($vr_1 = 50.01$, $p < 0.001$) and fighting was most

frequent in interval 1 (vr_4 = 107.16, $p < 0.001$) between unfamiliar pigs (vr_1 = 17.07, $p < 0.001$). After the initial period fighting was equally infrequent between familiar and unfamiliar pigs. Unfamiliar pigs were generally the targets of more attacks (vr_4 = 5.44, $p < 0.001$). However, littermates received more SAI after interval 1 (vr_4 = 3.98, p = 0.004) and overall (vr_1 = 25.85, $p < 0.001$). Although both sexes directed more SAI to familiar pigs, females directed even fewer SAIs to unfamiliar pigs in comparison with the males (mean frequency familiar:unfamiliar, males = 4.9:4.2, females = 4.6:3.3, vr_1 = 4.21, p = 0.041). More SNAI were directed towards unfamiliar pigs in the first interval (vr_4 = 7.73, $p < 0.001$), although the opposite was true from the second interval and overall (vr_1 = 15.16, $p < 0.001$). Unfamiliar animals were mounted more frequently in the first interval (vr_4 = 3.70, p = 0.012), but not after this or overall.

Table 3.3 Frequency of behaviours (interval mean) directed towards familiar (littermates) or unfamiliar pigs, by time interval, with significance level of familiarity and interactions between familiarity*time and familiarity*sex (Repeated Measures ANOVA, * p<0.05, ** p<0.01, *** p<0.001)

Behaviour (frequency)		Time Interval Mean by Familiarity 1	2	3	4	5	Familiarity	Familiarity* Time	Familiarity* Sex
Aggression	Familiar	11.2	6.9	3.7	3.6	3.6	P = 0.073	***	NS
	Unfamiliar	13.6	6.2	3.3	2.5	2.0			
Fights	F	0.6	0.2	0.1	0.1	0.1	***	***	0.084
	U	1.3	0.3	0.1	0.0	0.1			
Attacks	F	0.7	0.5	0.4	0.5	0.5	***	NS	NS
	U	2.2	1.3	0.9	0.7	0.6			
SAI	F	9.3	5.8	2.8	2.9	3.0	***	**	*
	U	9.5	4.2	2.0	1.8	1.2			
SNAI	F	12.7	9.8	5.7	0.6	0.7	***	***	NS
	U	15.2	7.8	3.6	0.4	0.4			
Mount	F	0.6	0.5	0.3	0.2	0.3	NS	*	NS
	U	1.0	0.5	0.2	0.1	0.1			

3.3.1.2 Lying preference

There was an effect of interval (vr_4 = 44.54, $p < 0.001$), choice of lying partner (vr_2 = 91.99, $p < 0.001$) and an interaction between time interval and lying partner (vr_8 = 11.95, $p < 0.001$), on the frequency of lying, but no effect of sex. Pigs were generally more likely to lie next to a familiar pig (littermate), followed by 'no pig' (Figure 3.2). Lying next to unfamiliar

pigs was the least frequent overall, although in intervals 2 and 3 unfamiliar partners were chosen as frequently as 'no pig'. Lying was least frequent in intervals 1 and 4.

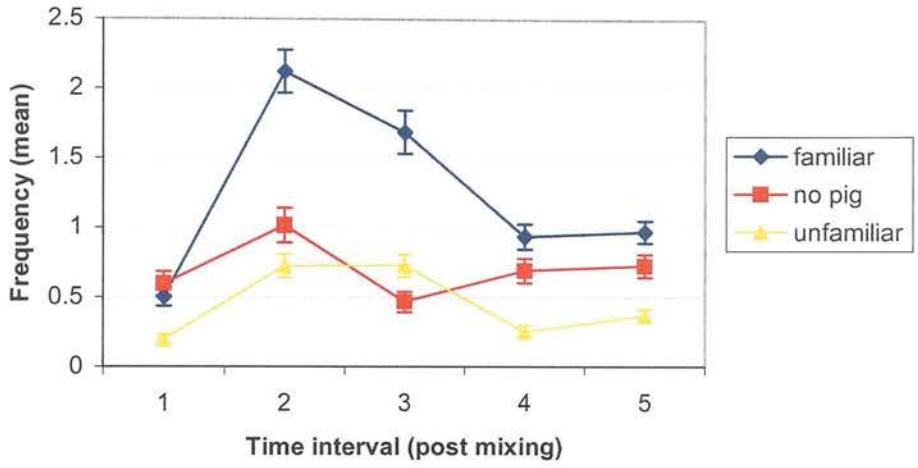


Figure 3.2 Frequency of lying by interval, with preference for lying next to a familiar pig (littermate), unfamiliar pig (other litter) or no pig (on own)

3.3.1.3 Mounting

Behaviours that correlated with the incidence of mounting were total aggression ($r = 0.162$, $p = 0.021$), attacking ($r = 0.187$, $p = 0.008$), fighting ($r = 0.140$, $p = 0.048$), chasing ($r = 0.161$, $p = 0.022$), contests won ($r = 0.192$, $p = 0.007$), received mounting ($r = 0.174$, $p = 0.014$) and received aggression ($r = 0.286$, $p < 0.001$). The low coefficients suggest that the relationships are not linear. Defining pigs by mean frequency of mounting, high mounters were more aggressive (t-test, $t_{198} = 2.74$, $p = 0.007$) and more likely to be the recipients of mounting ($t_{198} = 7.47$, $p < 0.001$). They were less likely perform the non-social behaviours lie, rub and avoid ($t_{187} = -2.44$, $p = 0.016$). Pigs that mounted during the weaning mix were more likely than would be expected by chance to mount during both RIT1 (26 %, $\chi_2 = 5.445$, $p = 0.02$) and RIT3 (52 %, $\chi_2 = 5.946$, $p = 0.015$) (Chapter 2).

3.3.1.4 Cortisol

Cortisol levels had increased by 30 minutes post mixing (Figure 3.3), although this rise was not significantly greater than the pre-mix baseline measure. Compared to the baseline, cortisol peaked 24 hours post mixing ($t_{89} = -2.17$, $p = 0.032$). Day 7 values were similar to baseline measures and were lower than both 24 hours ($t_{92} = 2.17$, $p = 0.008$) and 30 minutes post mix ($t_{88} = 1.90$, $p = 0.06$).

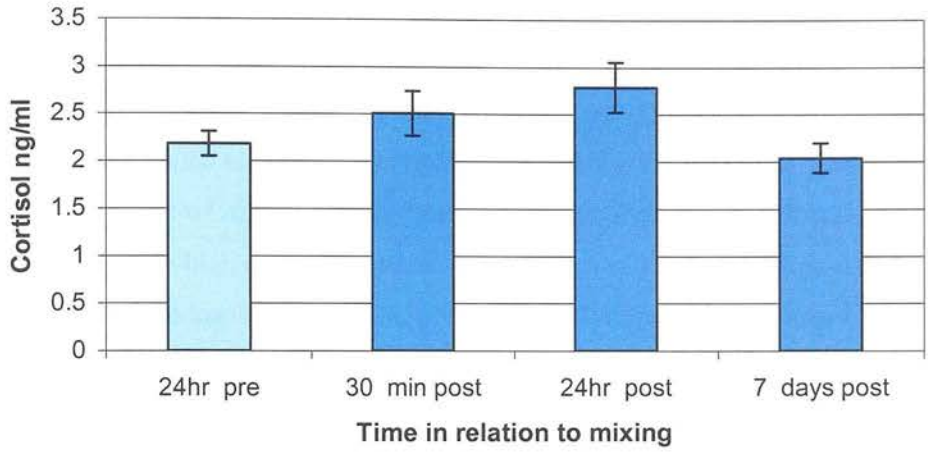


Figure 3.3 Mean (± SEM) salivary cortisol 24 hours pre-mix (baseline), 30 minutes after mixing, 24 hours and 7 days after mixing

Grouping pigs by mean baseline cortisol, low baseline pigs received more aggression (t_{92} = -2.54, p = 0.013) and had a greater gain in cortisol 30 minutes post mixing (t_{88} = 2.10, p = 0.039). There was a mild positive correlation between baseline cortisol and subsequent success in fights (r = 0.253, p = 0.014). Pigs that avoided other pigs more frequently, had lower cortisol gains from baseline to 24 hours (r = -0.335, p = 0.001) and lower cortisol 7 days later (r = -0.249, p = 0.016).

3.3.1.5 Lesions

Pigs in the high-receiving-aggression category tended to have fewer total lesions (mean lesions, high received aggression = 8.494, low = 9.121, t_{175} = -1.75, p = 0.066) and fewer severe lesions (high = 1.67, low = 2.27, t_{158} = -2.10, p = 0.037). Pigs more frequently fighting had a greater number of lesions (high = 9.2 lesions, low = 8.5, t_{175} =, p = 0.04). There were some weak associations between the number of lesions and behaviour frequencies. Attacking and biting (N = 177, r = 0.151, p = 0.045), fighting (r = 0.141, p = 0.061) and interrupting fights (r = 0.200, p = 0.007) were weakly correlated to total lesions. There was a weak tendency for more severe lesions in pigs that interrupted fights (r = 0.134, p = 0.076) and fewer severe lesions in pigs that chased others (r = -0.209, p = 0.005). Total and severe lesions did not correlate well with any of the duration measures of fighting, pushing and attacking. There was a mild correlation between total lesions and cortisol gain 30 minutes post mixing (N = 175, r = 0.233, p = 0.042).

3.3.1.6 Food competition test (dominance rank)

Dominance correlated weakly with the frequency of several aggressive behaviours (Spearmans $_{178}$, $p < 0.001$ for all), including; total aggression ($r_s = 0.242$), attacking ($r_s = 0.251$), fighting ($r_s = 0.272$), contests won ($r_s = 0.303$) and duration of aggression ($r_s = 0.268$). Dominance rank did not correlate with latency to aggression, SNAI, mounting, any of the measures of lesions, or RIT latency to attack. Pigs successful in the mix were more likely to have a high dominance score ($\chi_2 = 9.73$, $p = 0.008$), although there was no linear correlation. Dominant ($r_{s175} = 0.174$, $p = 0.021$) and successful pigs ($r_{s175} = 0.178$, $p = 0.018$) were more likely to interrupt fights, particularly fights involving an unfamiliar pig ($r_{s175} = 0.158$, $p = 0.035$). Heavy pigs were more likely to be dominant ($\chi_1 = 7.164$, $p = 0.007$), but this relationship was not clearly linear ($r_{s136} = 0.232$, $p = 0.002$) and was confounded by sex as males were heavier and more dominant than females ($\chi_1 = 12.919$, $p < 0.001$). Dominant pigs had greater cortisol 30 minutes post mix ($r_s = 0.212$, $p = 0.049$), but lower cortisol and lower cortisol gain 24 hours later (+24 hr, $r_s = -0.309$, $p = 0.002$; gain +24 hr, $r_s = -0.246$, $p = 0.017$) and 7 days later ($r_s = -0.228$, $p = 0.03$).

3.3.1.7 Resident Intruder Test (RIT)

Latency to attack in RIT did not correlate with latency to aggression in the mix. High and low mix-aggression pigs showed no difference in RIT1 attack latency, but high-mix aggression pigs were slower to attack in RIT3 (mean RIT latency seconds, high mix-aggression = 370.6, low = 331.8s, $t_{194} = 2.20$, $p = 0.029$). As sex affected attack latency in RIT3 (see Chapter 2), the analysis was repeated with males and females separately. High-mix aggression females appeared to be quicker to attack in RIT3 (high = 257.8, low = 281.7), although this was not significant; conversely high mix-aggression males were significantly slower to attack (high = 449.7, low = 415.8, $t_{87} = 2.18$, $p = 0.032$).

3.3.1.8 Weight, body dimensions and group size

Males were heavier than females (mean weight kg, females = 9.49, males = 10.42, t_{196} = -2.91, p = 0.004). Body dimensions and weight were highly correlated (r = 0.756, p < 0.001). There was a slightly better association between success in fights and body dimensions (r = 0.264, p = 0.002) than weight (r = 0.198, p = 0.02). There was no effect of weight asymmetry between mixed litters on aggression. Group size did not affect social behaviours, yet non-social behaviours (lying, rubbing and avoiding) were more frequent in larger mix groups (mean individual frequency in large groups = 17.4, small = 12.8, t_{198} = 3.24, p = 0.001).

3.3.2 Mix behaviour analysis – Principle Components Analysis (PCA)

The ethogram of weaning mix behaviours produced four factors/dimensions with Eigenvalues greater than 1. These principle components explained 60 % of the variation in the data (Table 3.4). A high frequency of attacking, fighting, biting and greater time spent attacking or fighting, defined the first dimension (21.2 % of variation), with longer latencies to perform aggressive behaviour at the other extreme. As attacking, biting and fighting were clustered together they were merged into a single behaviour category. The second dimension (16.5 % variation), was defined by high frequencies of play, chasing, short aggressive and non-aggressive interactions. The opposite axis of this dimension was less clearly defined and appeared to be fights with longer duration (fight pause, fight duration) and lying. The recipients of aggression and mounting, in contrast to lying defined the positive and negative axes of factor dimension 3. Dimension 4 described the non-social behaviours lying, rubbing and avoiding compared to short non-aggressive interactions.

Table 3.4 Factor loadings (Varimax Rotation) and percentage variation explained for factor dimensions 1 to 4 (Eigenvalues greater than 1) for PCA of mix behaviour frequencies and aggression duration. Behaviours with the greatest positive and negative loading value and behaviours with values greater than 0.5 (+/-) on each axis are indicated

Behaviour (frequencies)	Factor			
	1	2	3	4
Attack, Fight & Bite	**0.919**	-0.088	0.021	-0.043
Fight Pause	0.480	**0.395**	0.278	0.045
Chase	0.370	**-0.654**	0.166	-0.030
Fight Interrupt	0.487	-0.225	0.001	-0.015
SAI	0.341	**-0.800**	0.103	0.112
Mount	0.229	0.042	**0.556**	-0.046
Received Aggression	-0.095	-0.273	**0.786**	-0.048
Received Mount	-0.062	-0.063	**0.763**	0.120
SNAI	-0.040	**-0.630**	0.054	**-0.172**
Play	-0.192	**-0.810**	0.057	0.247
Rub	-0.031	-0.134	0.062	**0.745**
Lie	0.142	**0.133**	**-0.215**	**0.768**
Avoid	**-0.300**	-0.009	0.221	**0.550**
Duration of Aggression	**0.917**	0.131	0.115	0.012
Latency to Aggression	**-0.751**	0.081	0.095	0.096
Percentage variation	**21.2**	**16.5**	**11.7**	**10.6**

Loading plots were used to visualise patterns in the distribution of behaviours (e.g. Figure 3.4) and to assist interpretation of the factors. Clustering of behaviours and the degree of loading on a particular axis were used to assign descriptive titles; attacking (dimension 1), play (dimension 2), receiving (dimension 3) and lying (dimension 4).

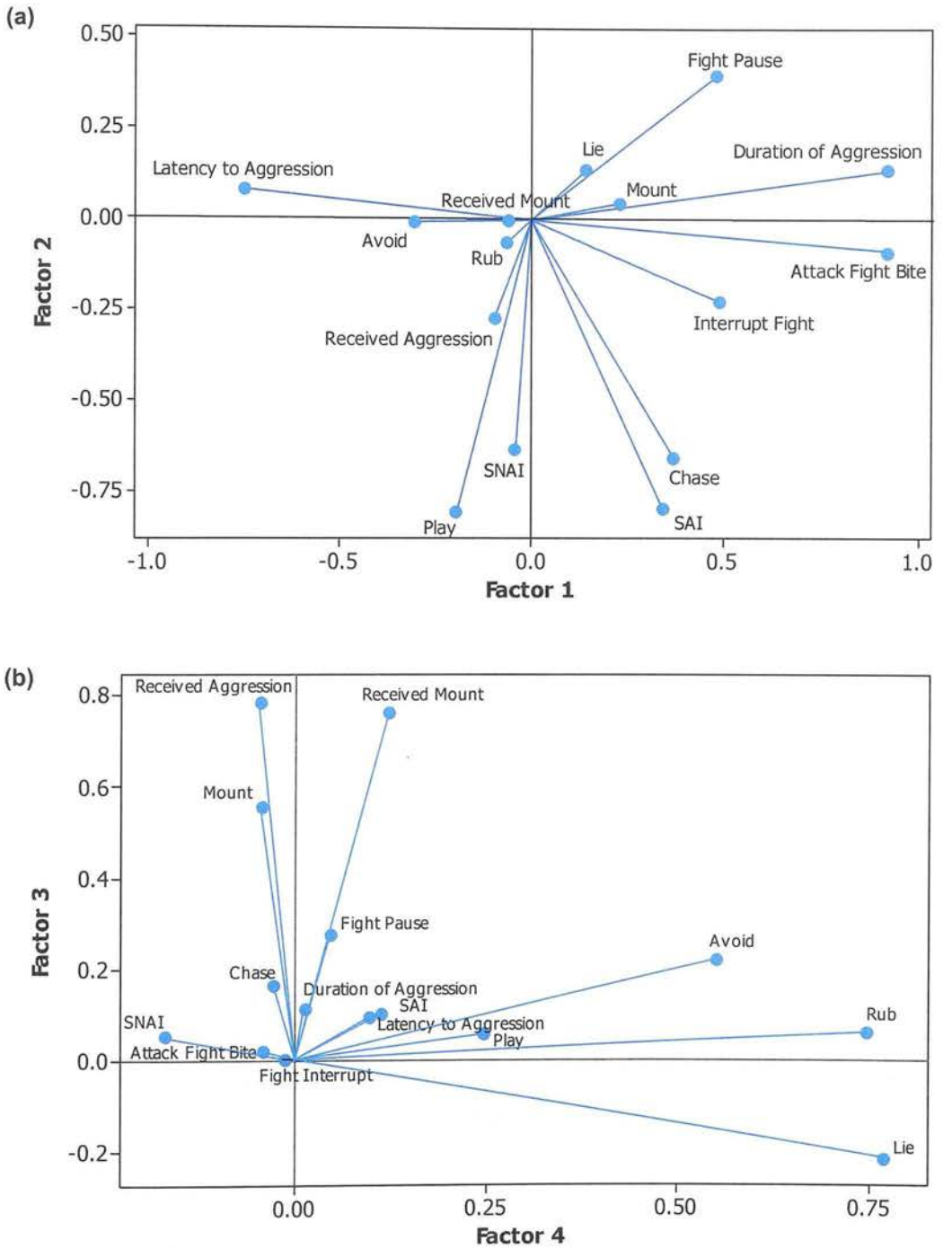


Figure 3.4 Loading plot of principle components (Varimax rotation), (a) dimensions 1 and 2, explaining 37.7 % of variation; (b) dimensions 3 and 4, explaining 22.3 % of variation in behaviour during the weaning mix

3.3.2.1 Mix behaviour and other measures (success, RIT, cortisol, lesions, weight and group size)

Highly successful individuals loaded on the attacking dimension (loading value 0.873), intermediate success pigs loaded on the opposite of the same dimension (-0.469) and low success pigs loaded in between (-0.046) ($F_{2,199} = 34.12$, $p < 0.001$). Latency to attack in the resident intruder tests did not load highly on any of the dimensions. Individuals with high lesion scores loaded with high attacking ($t_{171} = 2.60$, $p = 0.010$) and those with a greater number of severe lesions loaded with lying behaviour on dimension 3 ($t_{175} = -2.04$, $p = 0.043$).

Baseline cortisol loaded on the receiving aggression dimension (-0.462); pigs with lower baseline cortisol were associated with receiving aggression and higher cortisol associated with lying (high = -0.229, low = 0.329, $t_{92} = -2.52$, $p = 0.013$). High cortisol 30 minutes post mixing loaded equally highly with attacking (0.409) and low play (0.451). Measures of cortisol 24 hours after mixing and cortisol gain did not load highly on any dimension. Groups with higher than mean cortisol gain 24 hours post mix contained more pigs loading with frequent playing (dimension 2 loading, high cortisol gain = -0.327, low = 0.124, $t = -2.90$, $p = 0.004$). This was also true at the litter level (high gain = -0.257, low = 0.161, $t = -2.93$, $p = 0.004$), but not at the level of the individual pig. Higher cortisol 7 days post mixing loaded with lying behaviour, on the negative axis of the receiving dimension (-0.372).

Weaning weight did not load highly on the PCA dimensions. Loading scores were analysed by comparison to mean weight at weaning. Heavier pigs had scores associated with higher aggression (mean dimension loading, heavy = 0.177, light = -0.142, $t_{198} = 2.26$, $p = 0.025$), more play (heavy = -0.204, light = 0.164, $t_{198} = -2.63$, $p = 0.009$) and lying rather than receiving aggression (heavy = -0.143, light = 0.114, $t_{198} = -1.82$, $p = 0.071$). Group size loaded on dimension 4 (0.615), with non-social behaviours.

3.3.2.2 Individual mixing strategies

Figure 3.5 illustrates the spread of pig scores across dimensions 1 to 3. This spread was used to categorise pigs according to their behaviour in the mix. For example, pigs loading on the positive axes of all dimensions (+++) were associated with high attacking, low play and high receiving (see also Table 3.4).

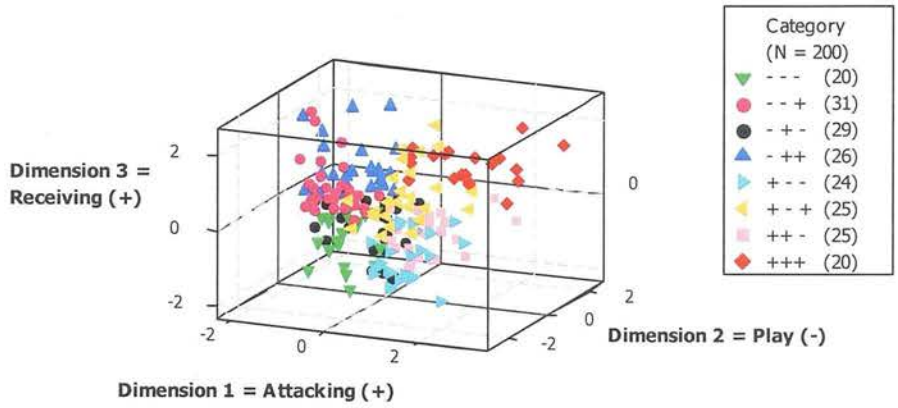


Figure 3.5 Three-dimensional matrix plot of pig scores on dimensions 1 (+ = high attacking), 2 (- = high play) and 3 (+ = high receiving). Categories were determined according to the sign (positive/negative) of scores on each dimension (Principle component factor analysis varimax rotation)

A contingency table was used to test for association between the categories and high/low rank (from the food competition test for dominance). Low ranking pigs did not exhibit high play or attacking in the mix (percentage of high play-low attacking pigs becoming dominant, 50 %; low play-high attacking 55 %; high play-high aggression 64 %; low play-low aggression 29 %, χ_7 = 20.303 p = 0.005).

The category with the highest cortisol increase 30 minutes after mixing (and highest gain from baseline) attacked frequently, did not play and received more aggression; those low in attacking and high in play had the lowest cortisol increase after 30 minutes (+30 min, F_{89} = 2.49, p = 0.023, +30 min gain, F_{89} = 2.68, p = 0.015). High attacking, low play, low receiving pigs tended to have the lowest cortisol gain 24 hours post mix (F_{198} = 1.97, p = 0.068). There was no difference between categories in lesions received.

3.3.2.3 Play versus aggression

Dimensions 1 (attacking) and 2 (play) defined aggression in the mix, and as described above, these aspects of behaviour seemed to define different mixing strategies between pigs. The analyses were simplified by grouping together categories to enable comparison of the extremes; pigs were classed as high attacking: low play (referred to as aggressive, n = 45), low attacking: high play (playful, n = 51). The remaining category included high play: high aggression and low play: low aggression ('other', n = 104). There was no significant difference between these categories in the quantity of aggressive acts displayed during the mix and there was no difference in ultimate dominance score (during the food competition test). 'Aggressive' pigs were involved in (ANOVA, $F_{197,2} = 12.64$, $p < 0.001$) and won more contests ($F_{197,2} = 24.24$, $p < 0.001$) (fights and pushing), but did not have any fewer undecided contests than 'other' or 'playful' categories. Whilst the 'other' and 'playful' categories of pigs were involved in the same number of fights, 'other' pigs won more of these. 'Aggressive' pigs were faster to perform an aggressive behaviour (mean latency, aggressive = 623.6 seconds, playful = 3878.0s, other = 3160.0s, ANOVA, $F_{199,2} = 19.38$, $p < 0.001$). There was a difference in mounting, with 'playful' pigs being the least likely to mount, then 'other', and 'aggressive' pigs being the most likely to mount (mean frequency, aggressive = 5.2, playful = 1.6, other = 4.1, ANOVA, $F_{199,2} = 3.90$, $p = 0.022$). There was no difference in likelihood or latency to attack in the resident intruder test.

'Playful' pigs generally appeared to have less lesions than the other 2 groups, but this was only significant for lesions to the ears ($F_{174,2} = 7.40$, $p = 0.001$). The 'aggressive' pigs had more lesions to the head ($F_{174,2} = 4.88$, $p = 0.009$), front ($F_{174,2} = 4.54$, $p = 0.012$), body ($F_{174,2} = 2.49$, $p = 0.086$) and overall ($F_{174,2} = 4.13$, $p = 0.018$) than both 'playful' and 'other' pigs.

There was no difference in baseline cortisol, 24 hours or 7 days post mixing. However, 'aggressive' pigs had higher cortisol 30 minutes after mixing ($F_{2,91} = 5.13$, $p = 0.008$) and a greater gain from baseline ($F_{2,91} = 3.26$, $p = 0.043$). 'Other' appeared to be higher than playful in both cases, but this was not significant.

There were more males in the 'aggressive' category (males to females, 33:12) and more females in the 'playful' group (15:36, $\chi_1 = 19.101$, $p = 0.000$), although there was no difference in weight between 'aggressive' and 'playful', or between males and females within these categories. There was a difference in the weight of male and female pigs in the 'other' category (males = 10.26,

females = 8.98, t_{93} = -2.87, p = 0.005), but not a difference in the relative proportions of males to females (51:53). There was a tendency for pigs of the 'other' category to be lighter on the day of mixing (other = 9.604, playful = 10.204, aggressive = 10.466, $F_{2,199}$ = 2.62, p = 0.075).

3.4 Discussion

3.4.1 Mix behaviour

3.4.1.1 Time, sex and familiarity

The duration and frequency of most behavioural measures were greatest during the first interval and typically declined over time. There were differences between sexes, with males being the more aggressive sex; they were faster to perform aggression, more successful in fights, and the frequency of various aggressive behaviours plateaued at a higher level in male pigs. Other studies have generally not found differences between male and female pigs in behaviour and relative aggressiveness. Nevertheless, in agreement with the results presented here, where differences have been noted it is often male pigs that either initiated more aggression, or spent more time fighting (Stookey & Gonyou, 1998; Giersing & Andersson, 1998; Colson et al., 2006). Interestingly, a study by Stookey & Gonyou (1994) found that it was female pigs that were more aggressive during mixing than males; conversely, the same authors found the reverse in a later study (Stookey & Gonyou, 1998), with males spending more time fighting than females. In both studies the situations were not directly comparable to here, as the males had been castrated (barrows). The more similar of the two was the later study (where females spent less time fighting) as mixing occurred at weaning; whereas, in Stookey & Gonyou (1994) the pigs were much older (average body weight approximately 85 kg) and were therefore approaching sexual maturity.

There was a second rise in some aggressive behaviours four hours post mixing, but again this was dependent on sex. Fight duration declined in females and the same was true of the males initially; however, fighting and attacking bout duration increased again in the males, without an increase in frequency. A second rise in aggression following mixing has been described elsewhere (Meese & Ewbank, 1973; D'Eath, 2002). This pattern of behaviour may be a consequence of high activity levels during the morning and afternoon, with a rest period in-between (Morgan et al., 1998). Meese & Ewbank (1973) found that different individuals were responsible for the second peak in aggression, but unlike here, no differences between sexes were reported. Differences between sexes in general activity and exploration in the home pen were found in a study by Bolhuis et al. (2006); however, no sex differences in social behaviour were reported, and this was also a comparison of females and barrows.

Receiving both aggression and mounting were unaffected by sex, as were short-non-aggressive and to a certain extent, short-aggressive behaviours. On the contrary, mounting and play were highly sex-linked; males were more likely to mount whilst females were more likely to play. This difference between the sexes decreased as the frequency to perform these behaviours declined. Newberry et al. (1988) found no significant sex differences in play and Dobao (1984/85) found that males played more. The inconsistency may be due to differences in the definition of what constitutes play. Dobao (1984/85) included pushing, butting, biting and mounting, whereas in this study all of these (except mounting) were classed as SAI. Distinct play behaviours (e.g. scampering, pivoting) were differentiated from acts that outside of play would be classed as aggression. In doing this it was possible to identify sex-differences that may have been indistinguishable otherwise. For example, SAI tended to be male-biased, as did mounting and so had these been combined with play then the extent of differences will have been masked. The reason for the differences between sexes in aggression and play cannot be fully explained, but these results do indicate that sex-differences should not be ignored when analysing behavioural data, even in pre-pubertal pigs.

Over time there was an increase in the duration, but not the frequency, of individual bouts of one-sided attacks. It is possible that this increase in unretaliated aggression, with a simultaneous decrease in mutual aggression (fighting/pushing), indicated the establishment of a dominance hierarchy. Similarly, D'Eath (2002) reported an increase in the frequency of one-sided attacks (referred to as bullying), but not fighting, in the afternoon following mixing.

The nature of aggressive behaviours differed according to the familiarity of the recipient (littermate or non-littermate). Unfamiliar pigs received more aggression, attacks, SNAI and mounting in the first interval, similarly Arey (1999) reported more fighting between unfamiliar pigs. Jensen & Yngvesson (1998) found that pigs spent less time nosing (equivalent to SNAI), but not less time biting, when re-exposed to an unfamiliar pig for the second time, suggesting that the nosing/SNAI behaviour is related to recognition. Unfamiliar pigs continued to receive more attacks after interval 1, but familiar pigs were the recipients of more SAI and SNAI (and possibly more mounting) from one hour after mixing. Aggression between familiar pigs was reported in Mount & Seabrook (1993) and it was suggested that mixing either disturbs established relationships, or that hierarchy formation does not prevent aggression. The amount of aggression received by familiar pigs in this

study only tended to be less than that directed to non-littermates, whereas there was a much lower frequency (6.6 %) in Mount & Seabrook (1993). One explanation for the difference is that the sows in Mount & Seabrook (1993) had recently been mixed into new social groups with previously unfamiliar pigs, before being mixed again and had therefore recently established a hierarchy. The weaned pigs in this study probably lacked the social experience of the sows and it is also possible that a hierarchy had not formed between littermates before weaning. Piglets will fight for possession of a particular teat when suckling (De Passille & Rushen, 1989) but there is little evidence to suggest that this relates directly to dominance (Litten et al., 2003). In support of this, pigs initially lacked the incentive to avoid other pigs, as they made little distinction between littermates and non-littermates when lying down, but after the intense aggression of the first hour they preferentially lay down next to littermates. Alternatively, the high levels of aggression directed towards familiar littermates may have been part of the frequently observed play-fighting sequences. Animals prefer to play with familiar conspecifics as play can involve self-handicapping, which leaves animals vulnerable (Spinka, 2001). From direct observation of mixing, pigs familiar to each other would often initiate play, although play (particularly scampering) seemed to be contagious and members of the other litter often joined in.

3.4.1.2 Cortisol

Cortisol levels were highest 24 hours after mixing and returned to baseline levels within seven days. There was a weak correlation between having higher baseline cortisol and greater success in fights. Additionally, pigs with lower baseline cortisol levels received more aggression and had a greater increase in cortisol immediately following mixing. In some species of primates it has been suggested that in non-violent societies with only occasional fighting (e.g. when a dominant position has been vacated) subordinate animals will have lower cortisol than dominants; in very aggressive societies, where stressors are frequent, subordinates will have higher levels (Abbott et al., 2003). If we consider those pigs that received more aggression as being more subordinate compared to those that won more fights, then this would fit with the proposed structure of the primate societies. After the initial fighting period, which the subordinate animals find more stressful, aggression becomes infrequent and the dominant animals once more have the higher cortisol levels. Similarly Merlot et al. (2004) found cortisol immediately post mixing was negatively correlated with success score; dominant/successful pigs had lower cortisol levels, but by 27 hours post mixing the reverse was true. They suggest that this is a consequence of greater negative feedback on the hypothalamic-pituitary-adrenal axis (HPA) in subordinate pigs.

There was another group of pigs, those that avoided others. These pigs had lower increases in cortisol post-mixing and lower cortisol several days after. It appears that there may be two kinds of subordinate animals. In rats these have been referred to as submissive and subdominant (Stefanski, 1998) and in pigs, low and no success; with low success pigs having the highest basal cortisol and greatest response to ACTH challenge (Mendl et al., 1992).

3.4.1.3 Lesions

Lesions were linked to aggressiveness. Pigs that received the most aggression tended to have fewer total and severe lesions, whereas pigs more frequently fighting had a greater number of lesions. Particular behaviours were associated with increased lesions, including attacking and biting, fighting and interrupting fights. Conversely pigs that chased others had fewer severe lesions. As with Turner et al. (2006) there was a link with involvement in fighting, but no connection between lesions and success. O'Connell et al. (2003) found low success in the nine hours post mix was correlated with a greater number of lesions, but this was after one week and not after just one day. As with Arey (1999) lesions were not correlated with dominance rank established during a feeding competition test.

3.4.1.4 Dominance and weight

There was a connection between aggression and success in the mix and subsequent dominance, although these relationships did not appear to be linear. There was no link between dominance, or latency to aggression in the mix and aggressiveness in the RIT (measured by latency to attack). Weight and sex confounded dominance score as heavy pigs were more likely to be dominant and males were heavier and more dominant than females. The link between weight and dominance has been widely reported (e.g. Drickamer et al., 1999; Jensen & Yngvesson, 1998), but a clear relationship between measures of aggression and dominance is less common. Fighting ability and subsequent dominance may result from a combination of various factors of which weight and aggressiveness are likely to be major contributing factors. Other circumstantial factors will influence the result of individual fights and as such, the winners of a particular contest may not necessarily be the most skilful (Rushen, 1990). Whilst many relationships may be determined in the period immediately following mixing, others may result from repeated contests over a period of time. In this study there would have been considerable opportunity for continued reassessment between the weaning mix and the dominance test pre-slaughter.

There were no differences in the frequency of aggressive behaviours between weaning groups of differing sizes (12 – 21 pigs). Andersen et al. (2004) found differences in aggressive behaviour between group sizes of 12 and 24 pigs, with more pigs fighting in the smaller groups. Space per pig was, however, kept constant, whereas in this study pen size remained constant and space allowance per pig varied. Turner et al. (2000) reported that more lesions (taken as a measure of relative aggressiveness) were counted on pigs with less space allowance; the equivalent here being the large weaning groups. Although increased aggression was not found in the larger groups this study, behaviours classed as non-social (lying down, avoiding and rubbing/rolling) were more frequent. There was no difference in aggression between mixes with greater or lesser weight asymmetry, although as litters were matched for weight wherever possible, the degree of asymmetry here was probably too small to cause an effect. The body dimension measures may have been a better predictor of success in the mix than weight alone, although any improvement was inconsequential.

3.4.1.5 Resident Intruder Test (RIT) - aggression and mounting

It has been suggested that aggression in the RIT is predictive of mix aggression (Erhard et al., 1997; D'Eath, 2002), but this was not found in this study. Pigs faster to attack in the mix were not faster to attack in the RIT; in fact high-mix aggression pigs were slower to attack in RIT3. This was influenced by sex and was probably in part confounded by the effect of mounting by males in RIT3 (see Chapter 2). In addition, females attacked faster in the RIT and yet these same female pigs were slower to attack in the mix immediately preceding the RITs. Faster RIT latencies in females pigs have been reported previously (D'Eath & Pickup, 2002; D'Eath, 2004), although in these studies no link was then made to mixing aggression. Although mixing and the RIT are both tests of social confrontation, it seems that the differences in methodology between them were too great to make them directly comparable. The RIT may not be functionally relevant in pig studies. The test was initially developed in rodents, where a dispute over territory between a single resident versus a single intruder, may simply be irrelevant in pigs. It could be argued that the RIT should still indicate which animals are inherently more aggressive; and strategies during mixing are likely to be influenced by aggressive personality. However, in the mixing situation pigs are better able to choose their opponents, and flee, or avoid particular pigs. They are also likely to be influenced by the presence of other pigs in the mixing situation; the presence of heavier male pigs for example, may have inhibited aggression in the female pigs.

There were several indications that mounting pigs were more aggressive, which confirmed the findings of Chapter 2. 'Mounters' in the mix also seemed to be the recipients of more mounting and were more likely to go on to mount in the resident intruder tests. Even though males were more likely to be dominant, dominance and mounting were unrelated. Both aggression in males and mounting behaviour may be under endocrine control as castrated males show reduced frequencies of both (Cronin et al., 2003).

3.4.2 Mixing - behaviour analysis (PCA)

Analysis of the weaning mix behaviours produced four factor dimensions explaining 60 % of the variation in the data. The two most dominant dimensions described overt aggressiveness such as fighting ('Attacking') and shorter, playful interactions ('Play') that included aggressive and non-aggressive behaviours. The other two dimensions were defined by receiving aggression ('Receiving') and non-social behaviours ('Lying'). The pattern of behaviours in the PCA alluded to different strategies employed by the pigs in the wean mix. Two particular strategies were examined further, playfulness and overt aggressiveness.

As would be expected, the aggressive individuals were involved in more fights and won more fights, but suffered more skin lesions. Playful pigs were generally least successful in fights, however this did not translate into lower ultimate dominance rank, in fact gaining higher dominance was connected to both play and aggression as low ranking pigs did not exhibit high levels of either. Male pigs out-numbered females in the aggressive category, whilst the playful group contained more females. D'Eath & Burn (2002) found that heavier pigs fought more and lighter pigs chased more. As chasing loaded highly on the play dimension and female pigs were generally lighter, a relationship with weight might be expected. Nevertheless the difference in strategy between play and aggression was not due to weight. There was no difference in weight between the categories, or between males and females within the categories. In contrast, there was no difference in the relative proportions of males to females in the 'other' category, but there was a difference in weight between the sexes in this category and in general 'other' pigs were lighter on the day of mixing. Mounting has been linked to play behaviour (see Newberry et al., 1988), but here it loaded with aggression. As discussed already, there was no connection between aggression and latency to attack in the resident intruder test.

The obvious question is whether the play response was simply motivated by the novelty and excitement or stressfulness of the situation, or whether it was a strategy employed by certain pigs in a mixing situation. Play does occur in response to novelty from a new environment (Spinka, 2001), increased space (Jensen & Kyhn, 2000) or the addition of stimulating substrates such as straw (Fraser et al., 1991; Dudink et al., 2006). It is also particularly prevalent in free-ranging pigs of the age studied here (Newberry et al., 1988). In addition, social interactions between same-age strangers are sought (Petersen et al., 1989) and so it is not inconceivable that the presence of the other litter might induce play. Play could function to counteract any negative emotions or stress from weaning, as it is generally considered to be rewarding (Spinka, 2001) or pleasurable (Fraser & Duncan, 1998). In support of this high-play-low-aggression pigs had the lowest rise in cortisol immediately post mixing. It has been suggested that play should only occur in safe conditions (Spinka, 2001). A mix does not seem particularly safe and Donaldson et al. (2002) found play was less frequent on the day of weaning compared to before weaning. Whilst play was not recorded in the period before weaning here, it was frequently observed after weaning. Those pigs that engaged in high 'playing' did suffer injuries, although injuries were not as severe as those suffered by pigs that engaged in high levels of overt aggression. In natural conditions fighting following introduction to the social group is rare (Newberry & Wood-Gush, 1986; Petersen et al., 1989), yet social orders still exist in free-ranging pigs (Graves, 1984; Jensen & Wood-Gush, 1984). One of the reasons for the lack of fighting could be an inhibition of aggression due to the presence of older, more dominant pigs, including the sow (Parratt et al., 2006). In the post-mixing observation period fights were more likely to be interrupted by a dominant or successful pig and so fighting may put pigs at risk of being attacked by higher status animals that they would not necessarily choose to fight. Play may be one method to assess relative fighting ability without overt aggression, particularly as it often includes aspects of serious aggression, including shoving, tossing head and circling (Newberry et al., 1988). The pigs that were playful did not attain any less status than the pigs that fought, although as behaviour was only monitored on the day of mixing it could be that these playful pigs reverted to aggression to gain rank in the days following the observation period. Thus, could play-type behaviour be a method to attain higher status without potentially costly fights? For example, this could be the case where pigs are not large enough, or sufficiently good at fighting, to confront a high ranking or aggressive individual; or alternatively, where overt fighting between pigs could result in either, or both, opponents subsequently being attacked by the most dominant pigs.

3.4.3 Conclusions

Differences were found between pigs in the performance of aggressive and non-aggressive behaviour during mixing at weaning. The two main descriptors of these strategies were playfulness and aggressiveness. Although aggressive and playful pigs did not differ in subsequent dominance rank when at pre-slaughter weight, they were more dominant than other pigs that had not been either aggressive or playful. As success during mixing was measured by conflicts won, aggressive pigs were by definition more successful than the playful pigs, as the aggressive pigs were involved in more fights and the playful pigs performed more short aggressive acts. The playful pigs received fewer injuries. Aggression occurred between littermates, although this took the form of short aggressive acts rather than the more severe attacks directed to non-littermates. One major and unexpected finding was that young male and female pigs behaved significantly differently in the period following mixing into new social groups; male pigs were more likely to follow an overtly aggressive strategy, whereas females were more likely to perform short aggressive and playful acts.

CHAPTER 4

AGGRESSIVENESS IN FEMALE PIGS, COMPARISONS BETWEEN BEHAVIOURAL STRATEGIES OF FEMALE PIGS MIXED AT WEANING AND AS GILTS

4.1 Introduction

Mixing unfamiliar pigs together elicits a period of intense fighting and aggression, resulting in elevated stress responses and injuries to the pigs involved (e.g. Mount & Seabrook, 1993; Arey, 1999; Ruis et al., 2002, and Chapter 3). This presents a particular challenge for the welfare of female pigs used for breeding. A common method for housing breeding sows was to keep them tethered in individual stalls, without the opportunity to perform normal social interactions with other sows. Although convenient for management, this practice has been shown to be detrimental to the welfare of sows (Wiepkema & Koolhaas, 1993; Janssens et al., 1994). Changes to European legislation now specify that sows must be housed in social groups from 2013[2]; a policy already adopted in the UK[3]. A consequence of this is that sows are therefore re-mixed into new social groups after each parity and are exposed to, or involved in, the ensuing aggression and associated problems. In 'dynamic' systems sows are kept in large groups with a constantly changing social structure; sows are continually added after weaning of their litters and pre-farrowing sows are removed (Durrell et al., 2003; O'Connell et al., 2004a). The potential for continual aggression is therefore much greater than in non-dynamic systems. As is sometimes the case, providing a solution for a particular welfare problem, such as individually housed sows, can present a different set of problems.

The impact of mixing on pigs at weaning and the different strategies employed by pigs of this age were discussed in Chapter 3. If pigs are consistent in their aggressiveness then there should be an association between relative aggressiveness during mixing as weaned pigs and during subsequent mixes. Consistency in aggression at mixing has been demonstrated in pigs over short periods of time (Mount & Seabrook, 1993) and over longer periods using the Resident Intruder Test (RIT) (Janczak et al., 2003a; D'Eath, 2004) and a group-feeding competition test (Ruis et al., 2000), but not between mixes over long periods. It is not clear whether the duration and severity of mixing aggression differs with age, particularly when comparing such a wide time period of piglet to pubescent pig. For example, many of the weaned pigs in Chapter 3 performed playful behaviour rather than serious fighting, but as

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[1] EU Legislation, Council Directive 2001/88/EC of 23 October 2001 amending Directive 91/630/EEC laying down minimum standards for the protection of pigs.

[2] Welfare of Farmed Animals (England) (Amendment) Regulations 2003 (S.I. 2003 No. 299).
The Welfare of Pigs Regulations 1991 Statutory Instrument 1991 No. 1477 (Scotland and Wales).

play is not common in adults these playful pigs would be expected to adopt different strategies as gilts. Age effects on aggression have been recorded in mice grouped as juveniles or adults (Bartolomucci et al., 2004a) and also in piglets mixed at various ages pre-weaning (Pitts et al., 2000) and immediately following weaning (Jensen, 1994). Changes in hormonal status and the escalating severity of injuries with increasing size are other factors linked to age that will probably alter the motivation to fight. In addition, sows and gilts will have superior social skills compared to weaned piglets, as they will often have been mixed at least once previously and social abilities appear to increase with experience (van Putten & Bure, 1997). If this is the case then mixing pigs as gilts may be less detrimental to their welfare, in comparison with mixing at weaning, as gilts should be able to settle disputes faster.

The aim of this experiment was to assess whether female pigs were consistent in aggressiveness across their lifetime. Consistency of aggressiveness, (i) within similar social confrontation tests, and (ii) across different types of social confrontation test was examined. In addition, (iii) the structure and severity of aggressive behaviour during mixing was compared, between observations of the same pigs as socially naive piglets and later as socially experienced gilts. Aggressiveness in the gilts was assessed through similar methods as detailed in Chapter 3 at weaning; a detailed analysis of mixing behaviour was performed and latency to attack an unfamiliar pig was recorded during a fourth RIT, when the gilts were 210 days old. This was then compared to data obtained for these same pigs during three RIT from 45 to 120 days of age (Chapter 2) and during mixing at weaning (Chapter 3). Analysis of the mixes used the same observation periods and ethogram. Salivary cortisol levels and lesion scores were also taken at the same points. Dominance ranks obtained from observations of food competition tests in the newly formed gilt groups were compared to a similar food competition test, performed on the pigs when in the weaning groups.

4.2 Methods

4.2.1 Animals and housing

The subjects were 36 gilts selected from the animals used in Chapters 2 and 3. The gilts had been raised in standard commercial conditions and remained in the same mixed-sex groups since weaning. When the pigs reached slaughter weight (approximately 90 to 110 kg live-weight) gilts were selected based on health status and leg-conformation. Where possible gilts were selected from the RIT treatment 123 (N = 32) so that the subjects had the same prior testing experience (weaning mix and three RIT). The remaining gilts (N = 4) were from treatments 13 and 23 (weaning mix and two RIT). Between two and three gilts were selected from each of 16 litters to create four mixed groups and two 'unmixed' groups. The mixed groups consisted of three sister-pairs that were from different weaning groups and were therefore unfamiliar. The unmixed groups contained six gilts from the same weaning groups, made up of two sets of three sisters; so they were all familiar to each other and related to half of the group. The gilt-groups were formed in straw bedded pens in the finisher accommodation. During this period they were fed ad libitum (Scotlean Prime Link, LC Rearer and Finisher Pellets, ABN). The gilt-groups were then moved into sow accommodation, consisting of a straw-bedded area, a dunging passage with a drinker and six separate sow-feeding stalls. Once in the sow accommodation food was restricted to one meal of dry pelleted feed (2.5 kg) every morning.

4.2.2 Procedures and measures

4.2.2.1 Gilt mix

Gilts were 175 days old (± 19.2 SD) when mixed. The pigs were numbered, weighed and body dimensions (height, girth at shoulders and crown-rump length) measured. The gilts were moved into the pen in their littermate pairs, with the youngest pairs moved first. Mixing took place between 1030 and 1130 hours. Behaviour was recorded continuously for five hours on the day of the mix from entry of the first pigs (colour digital and black and white VHS recordings). The video recordings were analysed using The Observer (v. 5.0.31, Noldus Information Technology, The Netherlands). The actor and recipient of behaviours were noted (ethogram Table 3.1) for the first 30 minutes (up to the saliva samples being taken) and for a further two hours, between

hours 1 to 2 and 4 to 5 post mixing. In the afternoon of the day following mixing (approximately 30 hours post mixing) fresh skin lesions for each body area (front, mid/flank, rear, and ears) on the left and right sides were counted.

4.2.2.2 Salivary cortisol analysis

Saliva samples were taken for cortisol analysis. Samples were taken at between 0900 and 1100 on the day before mixing (G-mix baseline), 30 minutes (G+30), 24 hours (G+24) and seven days (G+7) after mixing. The pigs voluntarily chewed the end of a large cotton bud, which was then placed in a salivette (Sarstedt) and spun in a centrifuge at 3000rpm for five minutes. The saliva was then decanted into cuvettes and frozen at –28 degrees centigrade. Analysis of the samples was performed by standard radioimmunoassay (COAT-A-COUNT® cortisol kit, TKC05, Diagnostic Products Corporation, UK) (Tunn et al., 1992). Samples, duplicates and standards were performed in the same assay; the detection limit was 0.475 ng ml^{-1} and medium intra-assay coefficient of variation was 8.6 %.

4.2.2.3 Resident intruder test (RIT)

The methods for this test have been described in detail in Chapter 2. In summary, a section of the home pen of the experimental animal (resident) was sectioned off and a smaller intruder introduced (mean 0.66 % of body weight ± 0.05 SD). The test was terminated when an attack occurred (rapid and persistent biting), or five minutes after the resident made contact (sniff) if there was no attack. The resident made contact with the intruder within 10 minutes of the intruder entering the pen on all test occasions. Following termination of the test the pigs were separated immediately. The pigs were considerably larger (210 days old, 158 kg ± 19.2 SD) than in the previous RITs and thus, the damage that they could inflict on each other, or on the stock-person trying to separate them, was potentially greater. So a jet of water was sprayed at the pigs to stop attacks and separate the pigs before the stock-person entered the pen. The water was effective at startling the pigs and stopping fights immediately, although they did not seem to find it unpleasant. At each testing point the resident pig was tested twice, once on each of two consecutive days, with a different intruder on each occasion.

4.2.2.4 Food competition test for dominance

A group feeding competition test was used as a measure of dominance status. There was no need to feed deprive the pigs as they were feed-restricted and therefore highly motivated to compete

for additional food. The test was performed approximately seven hours after the sows received their morning ration of food. The pigs were clearly marked and the dunging passage cleared of muck and straw. Two scoops of feed (approximately 2 kg) were placed in a line along the centre of the cleared area (1.5 to 1.75 metres in length). An observer recorded the actor and recipient of any 'attacks' (bites, knocks, pushing) and 'avoids' (the actor turned or ran away from the recipient without the recipient making physical contact). The test was terminated after all of the food had been consumed and two minutes after more than half of the group had lost interest in the area on the floor. The first test was performed one month after the gilts were mixed and was repeated on two further occasions, with at least two weeks in between. The gilts were assigned a dominance rank based on the number of pigs they dominated (attacks) and were dominated by (avoided), relative to total interactions.

4.2.3 Statistical analyses

The sample size for the analyses varied according to the test being analysed. All 36 pigs were mixed at weaning but as only 24 pigs were mixed as gilts then the data for comparisons between mixes used only the 24 mixed on both occasions. At the RIT, 32 pigs had experienced the test at all previous points (three occasions); the remaining four pigs had only received two RITs previously and were excluded (unless stated otherwise). The sample size for the analyses were: 24 for the gilt mix and comparisons between gilt, wean mixes and RIT; 36 for the fourth RIT; and 32 for comparisons across all of the RIT.

As in Chapter 3 the observation period (2.5 hours) was divided into five time intervals of equal length (30 minutes): from 0 to 30 minutes (interval 1); 60 to 90 minutes (2); 90 to 120 minutes (3); 240 to 270 (4) and 270 to 300 (5) minutes post-mixing. Data for individual pigs were also summed across the total observation period. Non-normal data were transformed (square root or natural log). Where normality was not achievable through transformation non-parametric statistics were used. Mean and median values quoted in the test, figures and tables were calculated from raw data. Cortisol was analysed by comparison to baseline values (24 hours pre-mixing). Baseline cortisol was also subtracted from the various test points to compare the magnitude of difference from baseline each point.

Parametric and Chi square statistical analyses were performed using Minitab (v.14) and Genstat (v.8) was used for the non-parametric analysis and Repeated Measures ANOVA. Differences between measures of the same animal (different parameters or time points) were analysed using paired t-tests, Repeated Measures ANOVA and 2-way ANOVA (blocking for pig). Equivalent non-parametric tests for paired data were Friedman's Non-Parametric ANOVA and Wilcoxon Matched Pairs Test. Pearson's (or Spearman's Rank) Correlation were used to test for associations between variables.

The RIT results were largely non-normal and so a combination of Wilcoxon Matched Pairs (to test for change) and Chi square (or Fishers Exact), Kendall's Coefficient of Concordance and Spearman's Rank Correlation were used. Comparisons across RITs 1 to 3 used a subset of data from Chapter 2 (as detailed above, data for 32 gilts).

Mix behaviours were analysed according to relative dominance. The pairs of sisters in the gilt mix were categorised using dominance ranks obtained in the dominance tests. The ranks

for sisters were added together and those with the lowest score were both classed as high-ranking, followed by mid ranking and those with the highest combined dominance score classed as low-ranking. These rankings were then used to compare differences in frequency/duration of behaviour as using One-way ANOVA and Tukey 95 % Simultaneous Confidence Intervals for pairwise comparisons. Non-normal data were compared using Kruskal-Wallis One-way-ANOVA and Mann-Whitney U test.

Comparisons between wean and gilt mixes used adjusted behaviour frequencies and durations. Wean mix groups consisted of between 12 and 21 pigs, whereas gilt mix groups contained only six pigs. In the larger mixes there would have been more opportunities for meeting and having aggressive encounters with 'unfamiliar' pigs. To account for the difference in group sizes the frequency and duration data for each pig was divided by the number of unfamiliar pigs. In the gilt mixes, the number of unfamiliar pigs was the same for all (one familiar sibling and four unfamiliar pigs). In the wean mix the number of unfamiliar pigs equated to the number of pigs in the other litter. This gave an estimated mean figure for the frequency and duration of behaviours directed per unfamiliar pig in each situation.

Finally, Principle Component Analysis (Varimax rotation) was used to compare gilt and wean mixes using the same behavioural ethogram. This analysis examined the relative importance of particular behaviours within mix, rather than a direct comparison of frequencies, which would be subject to the problems of varying group size described already. All behaviours that were independent of each other were included, except those that occurred infrequently in either or both mixes. The scores for individual pigs produced by these analyses were compared using a Pearson Correlation to look for relative consistency in behaviour between mixes.

4.3 Results

4.3.1 RIT – consistency and change

Of the 64 tests performed, 75 % (48) ended in an attack. There was no change in latency to attack between tests. Gilts experiencing the RIT for the fourth occasion in their lifetime (N = 32) showed some consistency in speed of attacking (test 4a and 4b, Spearman's correlation coefficient, $r_{s\ 30} = 0.367$, $p = 0.039$) and occurrence of attacking (attack yes/no, Fisher's exact, $p = 0.076$). This consistency was lost when the data for the four gilts tested only 2 times previously, were added ($r_{s\ 34} = 0.182$, $p = 0.288$; attack yes/no, $p = 0.413$).

4.3.2 RIT4 – comparison to RIT 1 to 3

Attacking was the most frequent outcome in all tests (attack rate, tests 1, 3, 4 = 75 %, 2 = 81 %). Of the 32 gilts tested at all eight points, 19 % attacked in every test and 44 % attacked at least seven times (Figure 4.1).

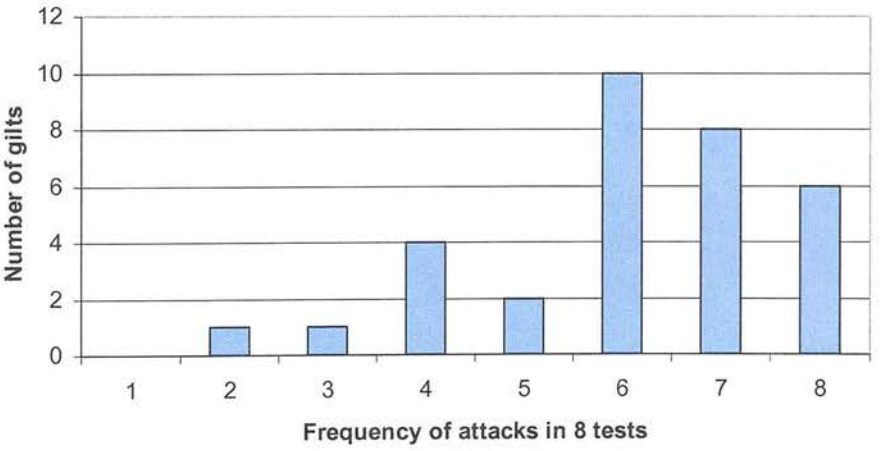
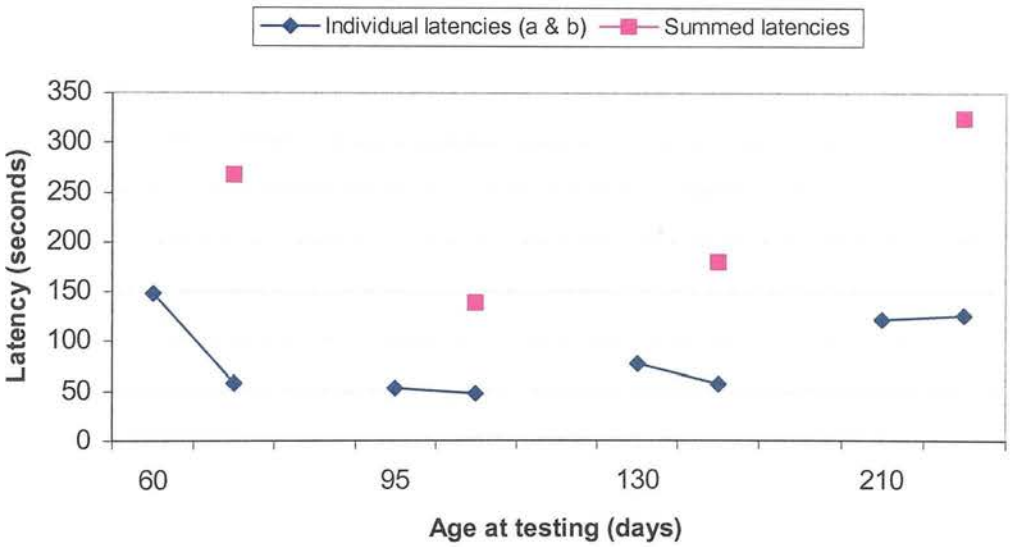


Figure 4.1 Frequency histogram of the number of attacks in 8 RITs for 32 gilts

The only difference in latency to attack between summed tests was between tests 2 and 3 ($W_{31} = -149.0$, $p = 0.050$) (Figure 4.2). Within test pairs the only significant difference was between tests 1a and 1b (Wilcoxon, $W_{28} = +84.0$, $p = 0.008$).

There was moderate consistency in the speed of attacking across the 4 summed test points from day 60 to 210 (Kendall's coefficient of concordance $_{31}$ = 0.444, p = 0.005) and rank-order consistency between RIT 1 and 3 ($r_{s\ 30}$ = 0.408, p = 0.020), 2 and 3 ($r_{s\ 30}$ = 0.429, p = 0.014) and a trend between 3 and 4 ($r_{s\ 30}$ = 0.313, p = 0.083). Within test-pairs the greatest consistency in speed of attacking was at 90 days (RIT 2, $r_{s\ 30}$ = 0.570, p < 0.001), followed by 120 days (RIT 3, $r_{s\ 30}$ = 0.414, p = 0.018) and 210 days (RIT 4, $r_{s\ 30}$ = 0.367, p = 0.039); relative attacking speed was not consistent at 60 days. There was evidence for pigs being either attackers or non-attackers within test, with significant associations, or tendencies, within all test pairs (1a & 1b, Fishers Exact, p = 0.033; 2a & b = 0.079; 3a & b = 0.014; 4a & b = 0.076). The only significant association between summed outcomes was between tests at 90 and 120 days (Fishers exact, p = 0.049).



	Range (seconds)							
	Day 60		Day 95		Day 130		Day 210	
	1a	1b	2a	2b	3a	3b	4a	4b
a & b	49 - 300	30 - 97	21 - 211	19 – 178	28 - 300	20 - 300	65 - 300	36 - 300
a + b	109 – 343		82 - 217		104 – 217		104 – 164	

Figure 4.2 Median latency to attack for 32 gilts during resident intruder tests 1 (day 60), 2 (95), 3 (130) and 4 (210), with a table detailing the range in values. For each test point, lines connect the separate values for individual tests (a & b) and summed values (a + b) are illustrated by single points

4.3.3 Gilt mix

During the 150-minute observation period short non-aggressive and aggressive behaviours were the most frequently performed (Figure 4.3) and there was a moderate relationship between these behaviours (Pearson correlation frequency SNAI & SAI, r = 0.409, p = 0.047). There was no difference in the frequency of attacking and mutual aggression (fight, push and pause), but attacking was more frequent than fighting (W = 22.5, p < 0.001).

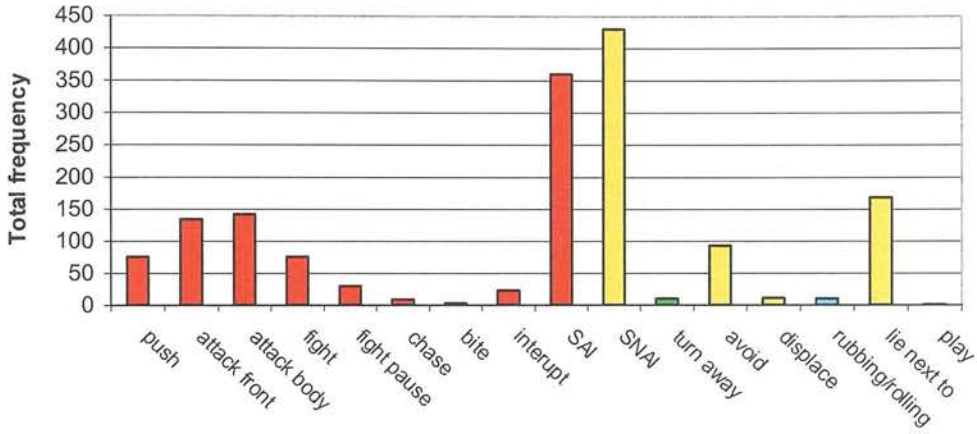


Figure 4.3 Total number of behaviours recorded during 150 minutes of observation, following mixing in 24 gilts, with colours indicating the different ethogram categories of behaviour: aggression (red), social (yellow), other/non-social (blue) and responses (green)

Pigs spent the same amount of time in mutual fighting as they did attacking (Figure 4.4). There was no difference in overall time spent in each of the separate elements of mutual (pushing, fighting) and one-sided aggression (attacking front or body), although individual fight-bout duration was longer than mean attack duration (median seconds attack = 7.25, fight = 18.71, W = 37.0, p < 0.009). Relatively little time was spent in pause or rub/rolling behaviour.

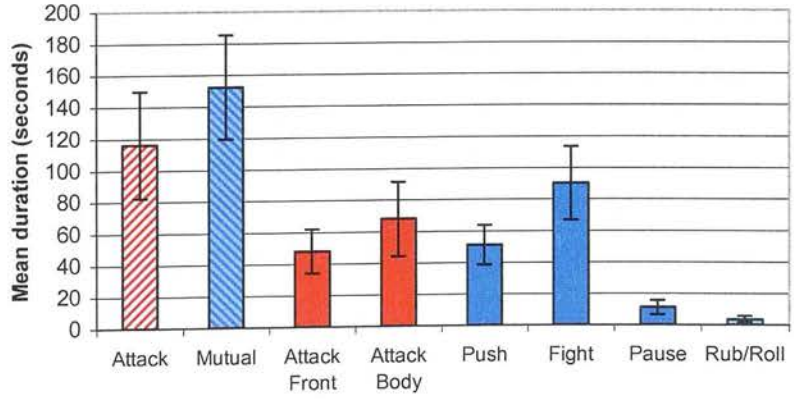


Figure 4.4 Mean total duration spent attacking (total; front & body separately), in mutual aggression (total; fight, pause & push separately) and rub/rolling, during an observation period of 150 minutes post-mixing in 24 gilts

4.3.4 Gilt mix – by interval

The frequency of most behaviours measured varied between observation intervals (Table 4.1); those that did not, included lie, avoid, displace, rub/roll and play.

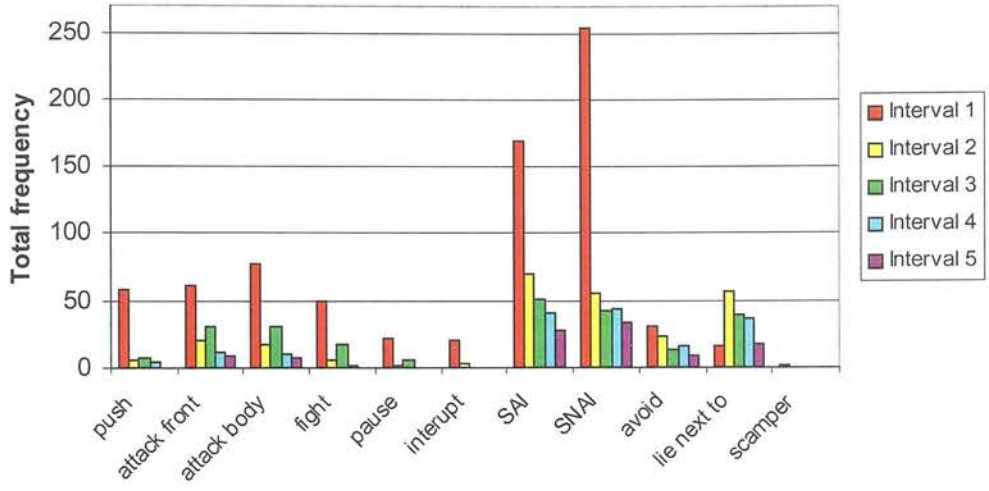
Table 4.1 Mean frequency and duration of behaviours recorded during intervals 1 (0-30 minutes post mixing), 2 (60-90 minutes), 3 (90-120 minutes), 4 (240-270 minutes) and 5 (270-300 minutes), with significance of time interval effect indicated (repeated measures ANOVA used for frequencies and Friedman's non-parametric ANOVA for duration data)

Behaviour	Interval					F - Value	Significance
Frequency	1	2	3	4	5	(df = 4, 23)	
Total aggression	18.42	5.46	5.88	3.04	1.96	22.11	<0.001
Attack	5.79	1.58	2.54	0.92	0.67	7.92	<0.001
Attack Front	2.54	0.88	1.29	0.50	0.38	5.71	0.001
Attack Body	3.25	0.71	1.25	0.42	0.29	8.04	<0.001
Fight	2.08	0.25	0.75	0.08	0.00	10.76	<0.001
Push	2.42	0.25	0.33	0.17	0.00	30.43	<0.001
Fight Pause	0.92	0.08	0.25	0.00	0.00	3.69	0.049
Interrupt	0.83	0.13	0.00	0.00	0.00	6.52	0.010
SAI	7.083	2.92	2.13	1.71	1.17	17.40	<0.001
SNAI	10.50	2.9	1.79	1.83	1.38	29.85	<0.001
Lie	0.58	2.38	1.67	1.54	0.71	20.51	<0.001
Duration (s)	1	2	3	4	5	(df = 4)	
Total aggression	151.70	28.90	63.30	18.55	6.03	42.53	<0.001
Total attack	52.41	13.69	33.47	10.5	6.04	19.95	0.001
Attack front	19.66	4.63	12.84	6.44	4.56	18.05	0.001
Attack body	32.75	9.06	20.60	4.07	1.47	18.63	0.001
Fight	52.40	9.23	22.88	5.42	0.00	28.42	<0.001
Push	38.90	5.13	4.51	2.63	0.00	49.06	<0.001

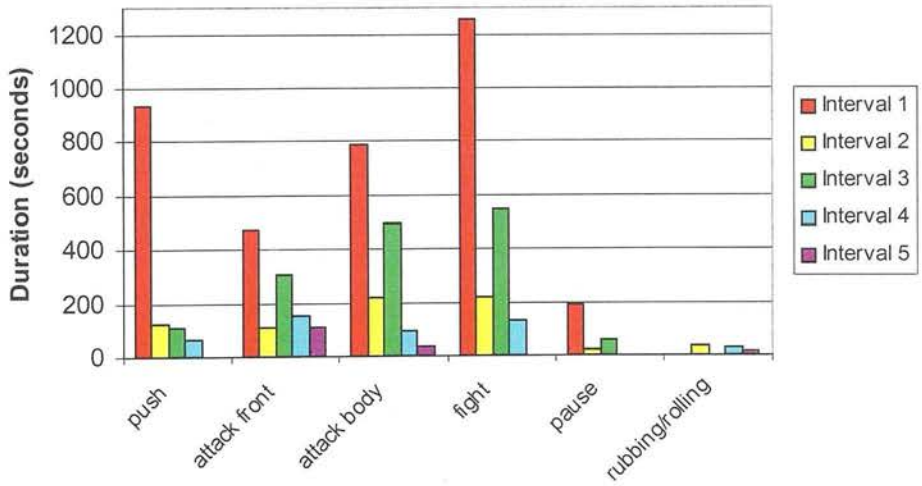
The majority of behaviours were most frequently performed during interval 1 and declined in subsequent intervals. Attacking and fighting showed a second, smaller increase in frequency and duration (Figure 4.5) during interval 3. The mutual aggression behaviours (fight, push, pause) and interrupting fights did not occur at all in the last interval. Lying was most frequent during interval 2.

Figure 4.5 Total frequency (a) and duration (b) of behaviours observed in intervals 1 (0-30 minutes post mixing), 2 (60-90 minutes), 3 (90-120 minutes), 4 (240-270 minutes) and 5 (270-300 minutes)

(a)



(b)



4.3.5 Gilt mix – lesions

The occurrence of skin lesions varied between pigs (ANOVA, $F_{23} = 12.89$, $p < 0.001$) and by their position on the body ($F_4 = 7.78$, $p < 0.001$) (Figure 4.6). There were more lesions to the front (head, ears, shoulder) than the body (rear, flank) (median front = 74.50, body = 30.00, Wilcoxon, $W_{24} = -27.50$, < 0.001).

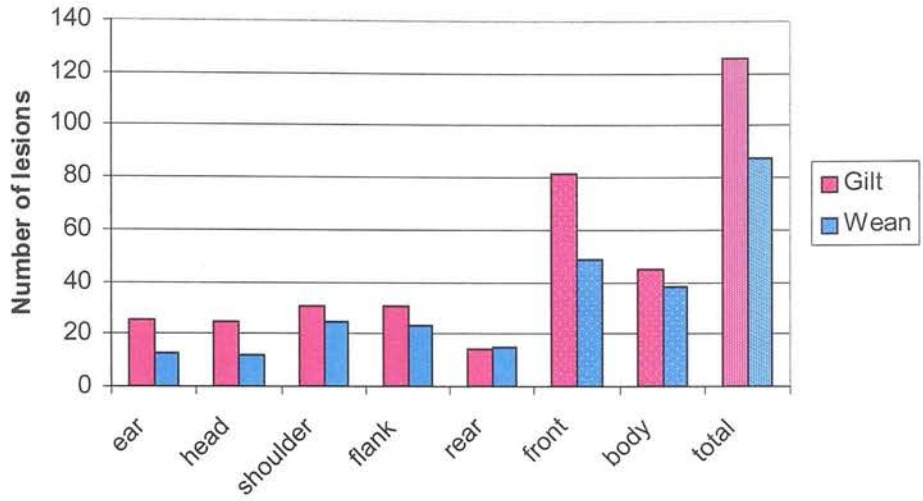


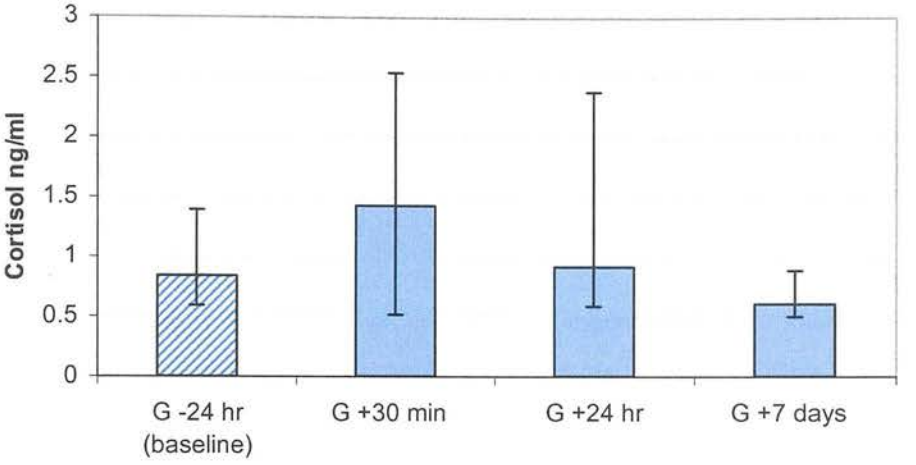
Figure 4.6 Skin lesions counted on 24 female pigs, after mixing at weaning and as gilts (at seven months old), by part of body affected, front (ear, head, shoulder), body (flank, rear) and overall total

Fighting and attacking were not directly related to total overall number of lesions, although pigs that fought did have a greater number of shoulder lesions ($r = 0.419$, $p = 0.042$). Pigs with more lesions performed less SAI ($r = -0.439$, $p = 0.032$), less SNAI ($r = -0.505$, $p = 0.012$) and interrupted less fights ($r = -0.433$, $p = 0.035$). Pigs that received more aggression did not have more lesions overall but did have more lesions to the rear ($r = 0.403$, $p = 0.051$).

4.3.6 Gilt mix – salivary cortisol

Cortisol levels compared to baseline values tended to be higher 30 minutes after mixing (median baseline = 0.84, +30 minutes = 1.43, Wilcoxon = -77.0, $p = 0.065$), there was no difference 24 hours post-mixing (+24, $p = 0.92$) and cortisol dropped below baseline seven days after mixing (+7 = 0.61, W = +54.5, $p = 0.010$) (Figure 4.7a). The change from baseline (Figure 4.7b) on day 7 was significantly different (and negative) to these earlier points (median change, +7 = -0.20, +30 = 0.25, W = -30.5, $p = 0.003$; +24 = 0.05, W = -34.5, $p = 0.011$). The change from baseline was not different between 30 min and 24 hours after mixing. Pigs with higher baseline cortisol had lower change from baseline 24 hours (Spearman's, $r_{s\ 22} = -0.64$, $p = 0.004$) and seven days ($r_{s\ 22} = -0.795$, $p = < 0.001$) after mixing, but not 30 minutes post-mixing.

(a)



(b)

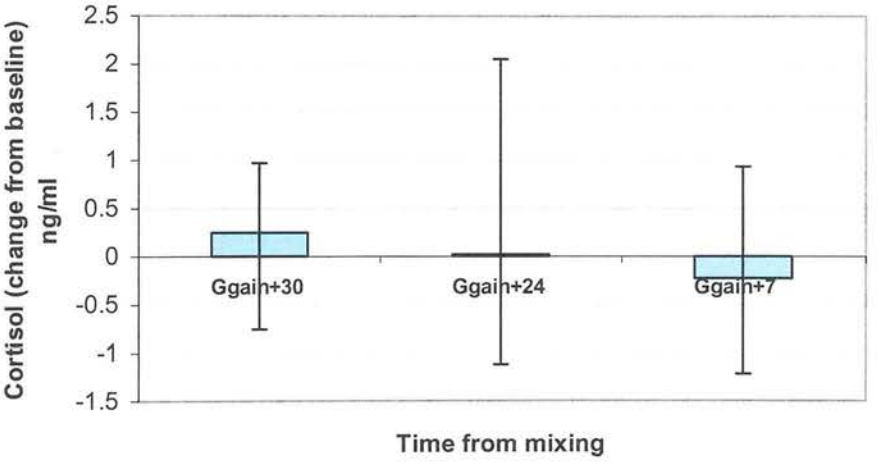


Figure 4.7 Median salivary cortisol levels, (a) 24 hours pre mixing as gilts (G-24) (baseline) and 30 minutes (G+30), 24 hours (+24) and 7 days (+7) post mixing; and (b) change from baseline, with interquartile ranges indicated

There were few behavioural correlates to salivary cortisol levels. Pigs receiving more attacks to the front tended to have higher baseline cortisol ($r_{s\,22} = 0.390$, $p = 0.060$) and a smaller rise (or greater decrease) in cortisol level from baseline seven days after mixing ($r_{s\,22} = -0.443$, $p = 0.030$). These pigs tended to receive more attacks to the body ($r_{s\,22} = -0.400$, $p = 0.053$) and lose more fights ($r_{s\,22} = -0.395$, $p = 0.056$). Using day +7 cortisol levels as a post-mix baseline cortisol level, pigs with higher cortisol had been avoided more during the mix ($r_{s\,22} = 0.441$, $p = 0.031$). This also tended to be the case when displacing others and being

avoided were combined as a composite measure of mix-dominance ($r_{s\,22} = 0.396$, $p = 0.055$). Additionally, higher day-7-cortisol-pigs tended to be faster to perform aggression ($r_{s\,22} = -0.370$, $p = 0.075$).

4.3.7 Gilt mix – by dominance

4.3.7.1 Behaviour

Pigs classed as high-ranking in the group-feeding competition tests following mixing, had attacked more frequently (Table 2), spent longer attacking, were more likely to perform SAI and SNAI, chase, win fights and interrupt fights during mixing as gilts. Pigs were least likely to lie next to these subsequently high-ranking pigs (mean high = 1.75, mid/low = 3.81, t-test, $t = -2.49$, $p = 0.021$). Pigs that were subsequently classed as low and mid-ranking received more aggression, were more likely to show submissive behaviours and tended to receive more SAI during the post-mix period.

Pigs classed as mid-ranking in the food competition tests appeared to be intermediate between high and low ranking pigs for many of the behaviours measured during mixing (e.g. total aggression, attack front and SAI). Some behaviours however, were performed equally infrequently by mid and low ranking pigs. These were, attack body, interrupt and SNAI. In addition, there was no difference between mid and low ranking pigs in received aggression or received SAI.

Involvement in mutual fights or mutual pushing did not differ with consequent rank (frequency or duration); neither did displacing others, latency to perform an aggressive act, or the number of SNAI received.

4.3.7.2 Lesions

Mid and low-ranking pairs had more lesions overall (means, high = 47.1, mid = 166.6, low = 166.4; ANOVA, $F_{23} = 5.40$, $p = 0.013$), on the front (h = 37.1, m = 103.9, l = 103.1; $F_{23} = 4.50$, $p = 0.024$) and body (h = 10.1, m = 62.7, l = 61.3; $F_{23} = 8.94$, $p = 0.002$) than the sisters ranked highest. The same pattern was also true for the individual body sections.

4.3.7.3 Salivary cortisol

There were no differences in levels of cortisol at any of the measurement points between the pairs of different rank.

Table 4.2 Frequency and duration of behaviours performed during the gilt mixes that were different according to pair-ranks (One-way ANOVA). Significant differences between means of high/mid/low pairs (Tukey Confidence Intervals) are indicated by cells containing different letters ($^{a \text{ or } b}$)

Behaviour	Mean frequency by pair rank			F-Value	Significance (df = 23)
	High	Mid	Low		
Frequency					
Total aggression	54.00^{a}	32.13^{ab}	18.13^{b}	8.80	0.002
Attack	21.13^{a}	7.63^{b}	3.75^{b}	11.44	<0.001
Attack Front	11.25^{a}	3.88^{ab}	1.63^{b}	7.80	0.003
Attack Body	11.88^{a}	3.75^{b}	2.13^{b}	8.62	0.002
Fights won	5.00^{a}	2.88^{ab}	0.25^{b}	10.02	0.001
Interrupt	2.50^{a}	0.25^{b}	0.13^{b}	7.18	0.004
Chase	1.00^{a}	0.13^{ab}	0.00^{b}	4.32	0.027
SAI	20.13^{a}	15.13^{ab}	9.75^{b}	4.42	0.025
SNAI	26.00^{a}	13.00^{b}	14.75^{b}	6.58	0.006
Received aggression	15.00^{a}	40.50^{b}	29.75^{b}	5.83	0.010
Received SAI	9.25	17.50	18.25	2.98	0.072
Submissive (avoid & displaced)	1.13^{a}	6.13^{ab}	7.00^{b}	4.44	0.025
Duration (sec)					
Total aggression	383.00^{a}	264.10^{ab}	124.40^{b}	3.87	0.037
Attack	239.00^{a}	75.30^{b}	34.00^{b}	7.46	0.004
Attack front	95.20^{a}	38.20^{ab}	10.95^{b}	8.74	0.002
Attack body	143.80^{a}	37.10^{b}	23.10^{b}	5.28	0.014

4.3.8 Gilt mix – comparison with weaning mix

4.3.8.1 Behaviour

Behaviour frequencies and duration were adjusted to account for the different number of pigs in the weaning and gilt mixes (see section 4.2.3). There was no difference in adjusted frequencies of attacking front, fighting (and duration) and receiving aggression between mixes. Gilts were faster to perform an aggressive behaviour (Table 4.3), they also spent more time performing the aggressive behaviours attacking and pushing, but not fighting. Attacking body was more frequent in the gilt mix, as were aggressive behaviours overall; however, SAI and SNAI were more common per unfamiliar pig in the wean mix. Dominance rank was not correlated between the mixes. Pause occurred at a low frequency in the gilt mix (adjusted

mean frequency = 0.31), but did not occur at all the weaning mix and play was very frequent in the weaning mix (adj. mean = 3.25), but the gilts did not play.

Table 4.3 Comparison of mean individual frequency and duration of behaviours in the wean and gilt mixes, adjusted for differences in mixing group size (frequency or duration divided by the number of unfamiliar pigs in each mix), Wilcoxon significance indicated

Behaviour	Mean adjusted frequency		Significance (N = 24)
	Wean mix	Gilt mix	
Frequency			
Total aggression	6.71	46.25	<0.001
Attack	0.51	1.63	0.026
Attack Body	0.08	0.50	0.002
Push	0.00	0.75	<0.001
SAI	5.40	3.63	0.056
SNAI	8.31	4.00	<0.001
Duration (sec)			
Total aggression	6.83	46.25	0.002
Attack	2.53	15.85	0.002
Attack front	1.94	7.06	<0.001
Attack body	0.42	3.45	<0.001
Push	0.00	8.47	<0.001
Latency to aggression	112.60	11.29	<0.001

4.3.8.2 Lesions

Lesions were more frequent in the gilt mix than the wean mix (mean individual lesion total, gilt mix = 126.04, wean = 80.63, Paired t-test, $t_{24} = 2.24$, $p = 0.035$). There was also a difference by body area, with more lesions to the front (ear, head, shoulders) in the gilt mix (medians, gilt = 74.5, wean = 44.0; $Wilcoxon_{24} = +59.00$, $p = 0.008$) but no difference between mixes in lesions to the body.

4.3.8.3 Salivary cortisol

Within the wean mix, cortisol 30 minutes after mixing was the only sampling point at which cortisol differed significantly from baseline (Wilcoxon = 71.0, $p = 0.023$). Cortisol did not differ between points 30 minutes, 24 hours and seven days post mixing.

Cortisol was greatest pre-weaning and was significantly higher than the pre-gilt-mix baseline ($W = -14.0$, $p < 0.001$). Between mixes cortisol did not differ at 30 minutes and 24 hours post (Figure 4.8), but did differ seven days after mixing ($W = -30.0$, $p < 0.001$). Change from baseline did differ between mixes at 30 minutes and 24 hours after mixing, with cortisol decreasing from baseline in the wean mix and increasing (or remaining the same) in the gilt mix (both points, $W = 61.0$, $p = 0.01$).

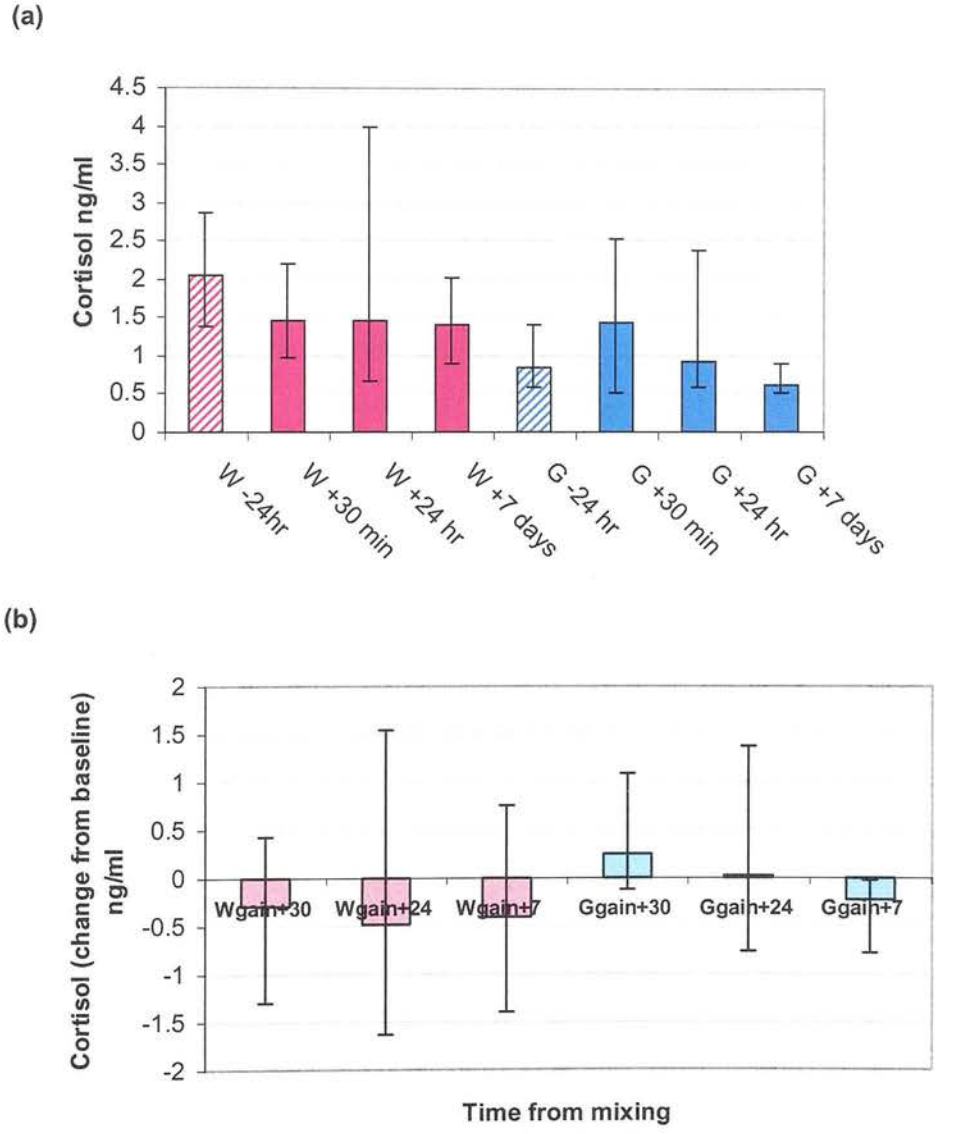


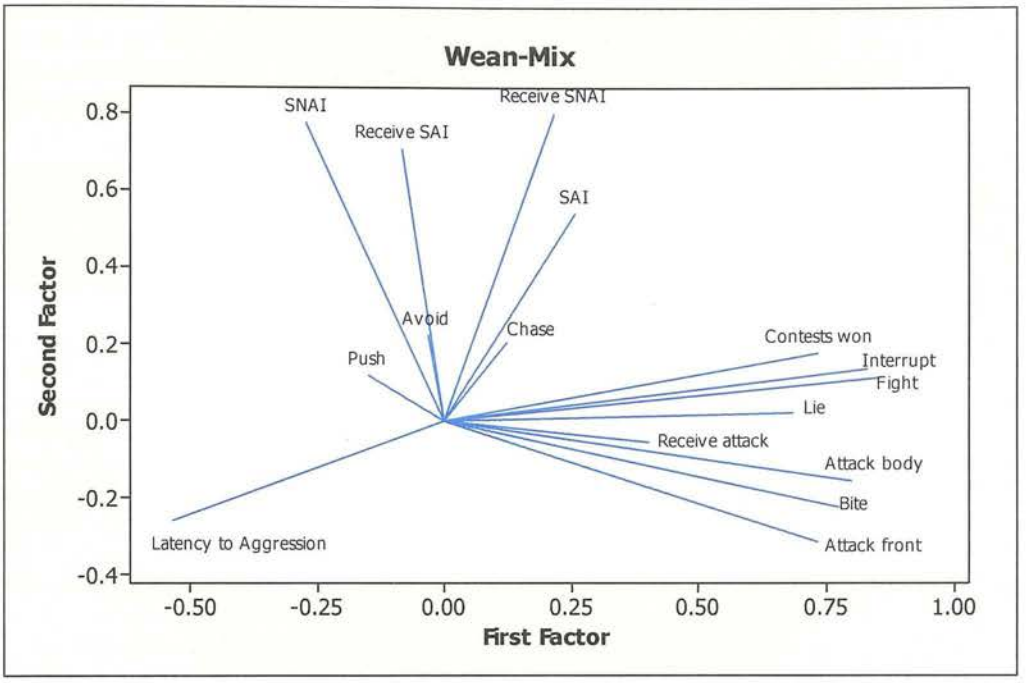
Figure 4.8 (a) Median salivary cortisol levels (baseline – 24 hours pre mixing, 30 minutes post, 24 hours post and 7 days post) and (b) change from baseline, for wean (W) mix and gilt (G) mixes, with interquartile ranges

4.3.9 PCA – individual behaviour patterns across mixes

Loading plots (Figure 4.9) illustrate the two factors in each mix that explain the most variation in the analyses. Play and pauses in fights were excluded from the analysis as they only occurred in one of the mixes.

The distribution and relative importance of particular behaviours differed between mixes. The most dominant factor dimension in the wean mix described aggression, with high frequencies of attacking and fighting at one extreme and long latencies to aggression at the other (Table 4.4). The second dimension described the short interactions (frequent short non-aggressive and aggressive interactions given and received) versus longer latency to aggression and more frequent attacking. In the gilt mix dimension 1 described aggression, but unlike the weaning mix, also included pushing and SAI; at the other end of the extreme were more frequent avoiding and receiving SAI. SAI and SNAI behaviours were less frequent than in the wean mix and did not dominate a particular axis. The second dimension in the gilt mix seemed to describe pigs that avoided and lay down frequently as opposed to those that took longer to be aggressive and fought more frequently. In both mixes the third dimension described the degree or nature of involvement in aggression, with receiving aggression (and avoiding in the wean-mix) at one end and shorter aggressive acts at the other (SAI at weaning, interrupting/chasing as gilts).

(a)



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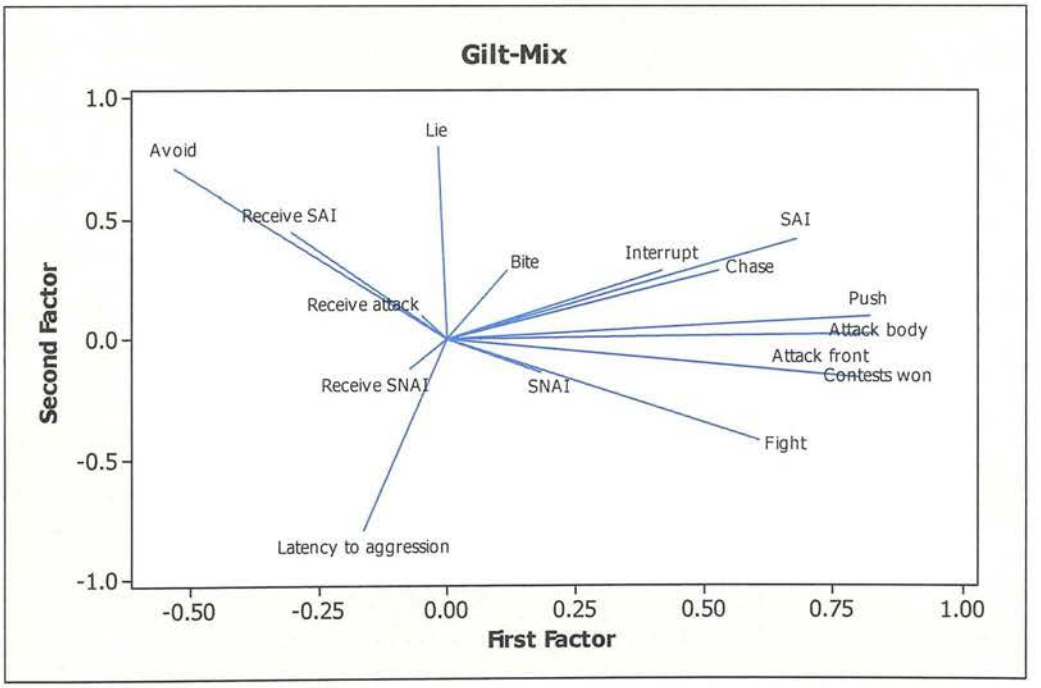


Figure 4.9 Loading plots of ethogram behaviours common to the weaning (a) and gilt (b) mixes, illustrating the two factors responsible for the greatest variation within each mix

Table 4.4 PCA factor loading scores for the same 24 pigs at two time points (mixing at weaning mix and as gilts) using the same behavioural ethogram. Pause was too infrequent to be included in the weaning mix and play was too infrequent to be included in the gilt mix and so both have been excluded. Behaviours with the greatest loading values on each axis are indicated

Behaviour	Weaning mix factors				Gilt mix factors			
	1	2	3	4	1	2	3	4
Push	-0.150	0.119	-0.415	**-0.738**	**0.820**	0.096	0.393	-0.061
Attack front	**0.734**	**-0.315**	0.182	-0.435	**0.726**	-0.150	-0.271	-0.232
Attack body	**0.798**	-0.155	0.096	-0.294	**0.833**	0.019	-0.317	0.009
Fight	**0.854**	0.116	0.173	-0.103	**0.608**	-0.424	0.106	**0.496**
Chase	0.125	0.205	**0.300**	**-0.692**	**0.527**	0.287	**-0.491**	-0.354
Bite	**0.776**	-0.223	0.064	0.011	0.115	0.284	-0.233	**-0.588**
Fights won	**0.736**	0.177	-0.243	0.106	**0.800**	-0.159	-0.247	0.113
Interrupt fight	**0.830**	0.138	-0.083	0.068	0.418	0.284	**-0.485**	-0.105
SAI	0.259	**0.541**	**0.335**	**-0.505**	**0.680**	0.418	-0.223	-0.334
SNAI	-0.272	**0.775**	0.098	-0.165	0.182	-0.138	-0.321	**-0.735**
Avoid	-0.033	0.224	**-0.619**	-0.023	**-0.532**	**0.713**	0.187	0.032
Lie	**0.685**	0.021	-0.309	**0.312**	-0.019	**0.795**	0.293	-0.077
Receive SAI	-0.082	**0.708**	-0.299	-0.364	-0.305	0.446	**0.672**	0.023
Receive SNAI	0.216	**0.801**	-0.113	0.076	-0.075	-0.126	0.231	**-0.782**
Receive attack	0.402	-0.053	**-0.699**	0.082	-0.051	0.093	**0.844**	0.113
Latency to aggression	**-0.535**	-0.261	**-0.588**	0.106	-0.162	**-0.795**	0.162	-0.076
Percentage variation	30.6	15.3	12.1	11.6	26.7	16.7	15.3	13.1

There was a moderate relationship between mixes in the pig scores for the second dimensions (Figure 4.10) (Pearson, r = -0.547, p = 0.006); pigs performing and receiving more short behaviour interactions in the wean mix (SAI and SNAI), took longer to perform an aggressive behaviour in the gilt mix. There was a weaker correlation between wean factor one (aggression) and gilt factor three (r = -0.400, p = 0.053). Pigs scoring on the axis for high frequency of aggression versus longer latency to aggression in the wean mix, were positioned with chasing and interrupting fights (with longer latency to aggression also at the other extreme) in the gilt mix analysis.

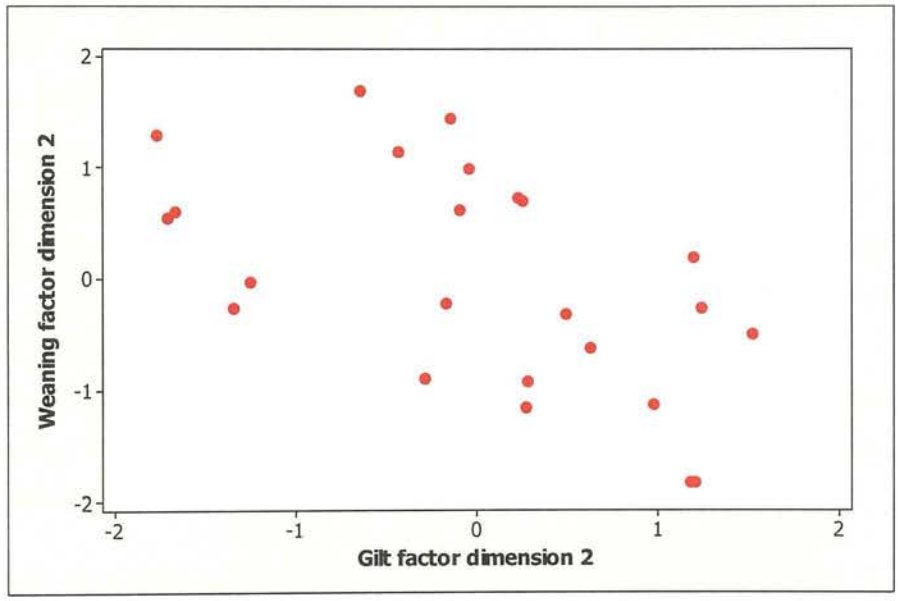


Figure 4.10 Plot of pig scores for the second dimension in the weaning and gilt mixes (r = -0.547, p = 0.006)

4.4 Discussion

4.4.1 Resident Intruder Test (RIT)

The results of the RIT on the pigs as gilts were similar to those performed during the growing phase (weeks 6, 13 and 17 post birth, Chapter 2). Gilts experiencing the RIT for the fourth occasion in their lifetime (30 weeks) showed consistency in the speed of attacking within and between tests. The only notable change in latency to attack was after the very first RIT (1a), probably as a result of novelty. Even a gap of 90 days between tests 3 and 4 (including the onset of puberty) did not alter the speed of attacking significantly. Gilts were consistent attackers or non-attackers within test. However, this relationship was less clear between tests. Attacking was by far the most frequent outcome (75 % to 81 %) and of the 32 gilts tested on all eight occasions, 20 attacked at least seven times. This confirms the results of Erhard & Mendl (1997), Janczak et al. (2003a) and D'Eath (2004), where pigs demonstrated consistency of responses across RIT at 7 and 11 weeks, 8 and 24 weeks and 6, 11 and 16 weeks respectively. However, unlike the studies by Erhard et al. (1997) and D'Eath (2002), this study found no link between relative aggression in the RIT and mixing aggression. This was also the case when comparing the pigs during mixing at weaning with RIT aggressiveness; as already mentioned (see Chapter 3) it appears that the two tests of aggressiveness are not directly comparable.

4.4.2 Gilt mix and comparison to weaning mix (Chapter 3)

4.4.2.1 Behaviour

There were some similarities between mixing at weaning and at 25 weeks of age (gilt mix). As found in the weaning mix (Chapter 3), the first 30 minutes after mixing was generally when behaviour frequencies peaked, followed by a decline over subsequent intervals. Short-non-aggressive and short-aggressive interactions were the most frequently performed behaviours. Attacking and fighting escalated again during interval 3 (2.5 to 3 hours post-mixing), although this second peak was smaller in frequency and duration. A second rise in fighting and attacking behaviour was also seen in the male pigs in Chapter 3, but not in the females; the only increase in female pigs was in attack bout duration. As detailed in Chapter 3, this second rise in aggression has been documented before (Meese & Ewbank, 1973; D'Eath, 2002), although the difference between the sexes has not been reported previously. If fighting functions to establish a social dominance order then the ratio of time spent in one-

sided attacking as opposed to mutual fighting should increase as the group-order settles. This is different from group cohesiveness, which may be better measured by how synchronised groups are in their behaviours (Merlot et al., 2004), or whether pigs no longer show discrimination in lying partners (Erhard et al., 1997; D'Eath, 2002). In studies of mixing aggression, fighting frequency and duration do generally appear to decline with time and aggression becomes more one-sided as 'loser' pigs are less likely to retaliate (Rushen & Pajor, 1987; Arey & Franklin, 1995). This process also seems be more rapid in pigs with greater social experience (van Putten & Buré, 1997; D'Eath, 2005). The gilts had received several opportunities to gain social experience from mixing at weaning and from repeated RIT; therefore it would be expected that the change from fighting to one-sided attacking would occur sooner and also that fighting would cease earlier in the gilt-mixes. This did appear to be the case, as by interval 2 the duration of attacking was greater than fighting in the gilts, whereas this did not occur until interval 4 in the weaning mix. In addition, the mutual aggression behaviours (fight, push, pause) and interrupting fights did not occur at all in the last interval of the gilt mix, but were recorded in the weaning mix. An alternative explanation for aggression ceasing earlier in the gilt mix is that as group size was smaller (six pigs) in the gilt mix the number of unfamiliar dyads would have been less and therefore social order may have been decided with fewer fights. To try and adjust for this, duration and frequency of behaviours per unfamiliar pig were analysed. This found that there was no difference in frequencies of attacking front or fighting. However, gilts were faster to perform an aggressive behaviour and spent more time attacking and pushing, but not fighting. Individuals performed more aggression overall as gilts, including attacking body, which is a particularly severe form of unretaliated aggression as the receiving pig will often be fleeing or trying defend itself from attacks to the head and ears (Rushen & Pajor, 1987; McGlone, 1985). Additionally, this one-sided attacking causes more lesions per second than fighting (Turner et al., 2006). Lesions were more frequent in the gilt mix than the wean mix. There was also a difference in where on the body lesions occurred, with more lesions to the front of the animals when mixed as gilts. This is indicative of mutual fighting behaviour (Turner et al., 2006) and suggests that although the gilt-mix may have been resolved faster it appeared to contain more severe injurious behaviour and more one-sided 'bulling' aggression (D'Eath, 2005).

Pausing occurs during long or strenuous fights and it was recorded in the gilt but not the weaning mix. Furthermore, play was very frequent in the weaning mix but did not occur in the gilt mix. These differences in the occurrence of particular behaviours between mixes,

suggests that the behavioural structure of mixing changed and these differences were highlighted by the PCA analysis. The most dominant factor dimension in both mixes described aggression. The second dimension of the wean-mix represented short interactions, whereas these behaviours did not dominate a particular axis in the gilt-mix and SAI loaded with aggression. The second dimension in the gilt mix seemed to describe pigs that avoided fighting and in both mixes the third dimension described receiving aggression. Although SAI and SNAI were the most performed behaviours in the gilt mix they did not dominate a dimension of the PCA and the playful scampering associated with these behaviours at weaning did not occur. However, even though the descriptors of dimension 2 appeared different, there was an association between them; pigs that performed more SAI and SNAI at weaning took longer to perform an aggressive behaviour in the gilt mix but were less likely to lie down and avoid others. The playful pigs at weaning may have adopted a strategy of low aggression but also low avoiding in the gilt mix. In the weaning mix playful behaviour may therefore have been a less serious, covert, form of aggression. As play does not occur very frequently in adult pigs then the equivalent strategy was to behave in a manner that is not overtly aggressive or submissive. The pig scores for first dimensions, although both describing aggression, were not correlated, neither were rank-order levels of attacking or fighting for individuals. So no direct link between relative aggressiveness was found between mixing at weaning and approximately 26 weeks later, as gilts, using the same behavioural ethogram. Otten et al. (1999) found some consistency in aggression with a period of 2-3 weeks between tests in pigs of about 12 weeks old and Hessing et al. (1993) found that pigs classified as aggressive at one week old were more aggressive in mixes at 10 and 15 weeks old. The lack of consistency in the present study may have been due to the differences in the behavioural structure of the mixes (e.g. presence of males) making them not directly comparable. Aggression in the females may have been suppressed at weaning due to aggression being male-dominated at this point. Alternatively, there was approximately 25 weeks between tests and the gilts had either been through or were going through puberty. In studies of human personality, although there is some consistency from childhood, levels of consistency increase with age into adulthood with some traits being more consistent, or more flexible, than others (Roberts & DelVecchio, 2000). It may be that aggression at mixing in pigs is a trait that shows greater long-term consistency in adult, rather than juvenile, pigs.

4.4.2.2 Lesions and dominance

The number and position of skin lesions varied according to behaviour in the mix, with fighting linked to having more shoulder lesions. Gilts that performed more SAI and SNAI had fewer lesions overall. Similarly at weaning, the playful pigs (high in play, SAI and SNAI, Chapter 3) seemed to have fewer lesions than aggressive pigs. However, unlike in the weaning mix, pigs that interrupted more fights, which could be considered a particularly risky and aggressive behaviour, also had fewer lesions. This effect was probably due to the social dominance order, which appeared to form more quickly in the gilt mix. Ultimately high-ranking pigs were more aggressive and yet had fewer lesions. These high-ranking individuals spent longer attacking, were more likely to perform SAI and SNAI, chase, win and interrupt fights and they were the least preferred lying partners.

SNAI encompassed all of the nosing behaviours (nose-to-nose, or nose-to-face and nose-to-body), although from observing the post-mix period, it would seem that most of the SNAI recorded were nose-to-nose or nose-to-face interactions. Jensen (1982) interpreted nose-to-nose as a threat behaviour, as it was more likely to be performed by dominant animals, with nose-to-body performed by subordinates. This might explain why the most dominant and aggressive pigs in the gilt mix were more likely to perform SNAI, which in the weaning mix was not correlated with aggression.

Low and mid-ranking pigs received more aggression, were more submissive (avoided and were displaced more) and had more lesions. As would be expected, mid-ranking pigs were intermediate between high and low ranks for many behaviours. They were however equally as unlikely as the low ranks to perform very dominant behaviours such as attack body, interrupt fights and perform SNAI. Several behaviours were unrelated to subsequent dominance and these included fighting, receiving SNAI and latency to perform aggression. Rank in the gilt mix was not correlated with dominance in the original weaning-mix groups. The group dynamics at these two points may have been too different (e.g. differing groups sizes and the presence of male pigs) to allow a direct comparison.

4.4.2.3 Salivary Cortisol

Cortisol increased 30 minutes after mixing, but returned to baseline levels by 24 hours post mixing and had dropped below baseline seven days after mixing. Pigs with higher cortisol seven days after mixing had generally been more dominant during the mix and faster to perform aggression. Whereas pigs with higher pre-mix cortisol received more attacks and

lost more fights, had a lower increase in cortisol 24 hours post-mixing and had lower cortisol seven days later. This corresponds with Merlot et al. (2004), as dominant/successful pigs had lower cortisol immediately following mixing, but by 27 hours post the reverse was true. The larger increase in cortisol in successful pigs was also reported in Otten et al. (1999). Greater reactivity in the HPA axis following stressors has been noted as a characteristic of less aggressive animals (Ruis et al., 2000) and so a larger increase post mixing might have been expected in this category. It is possible that negative feedback impacted on the results and it would require a greater number of sampling points to be able to discount this possibility (Smith & Dobson, 2002).

As with the gilt mix, the largest difference from baseline in the mix at weaning was at 30 minutes post mixing, but conversely, this was in the opposite direction: cortisol was lower than baseline in the wean mix and higher in the gilt mix. This situation only arose when analysing the weaning data for just the subset of 24 gilts. A large pre-weaning peak in cortisol was revealed, which had not been found when the data for all 94 male and female pigs were analysed at this point (Chapter 3). The exact reason for this is unclear. It may have been due to differences between sexes, or a chance subset of individuals that reacted more to the sampling procedure (this was the first saliva sample taken).

4.4.3 Conclusions

Female pigs were consistent in their aggressive responses from shortly after weaning to puberty, both within and across RIT. The speed of attacking did not change in female pigs beyond the first test point at 45 days old. Consistency across types of social confrontation test was not found. The RIT was not predictive of mixing aggression. In addition there was little evidence for consistency in aggressive behaviour between mixes, although the structure of the two mixes differed and may not therefore have been directly comparable. There was an association between mixes in non-aggressive behavioural strategies; the pigs that were playful at weaning seemed to be non-aggressive and non-submissive as gilts. Generally the gilt-mix was more serious: the short playful behaviours at weaning became associated with aggression, one-sided aggression was more frequent and the number of injuries increased. During the post-mixing period very clear differences were found between pigs that subsequently differed in rank: with differences in aggressive behaviours, cortisol levels and injuries received. In addition to the clear link between aggression and dominance-rank in the gilt mix, fighting ceased sooner compared to at weaning, suggesting that social order formed

faster in the pigs when they were gilts. The gilts were more socially experienced and their social skills may have been enhanced through previous mixing experience and repeated RIT. Even though fighting in the gilts was resolved faster, it did however appear to be more severe and injurious.

CHAPTER 5

DOES HAVING AN AGGRESSIVE PERSONALITY EFFECT MATERNAL ABILITY?

5.1 Introduction

The quality of maternal behaviour and the reaction of sows to the farrowing environment have important consequences for the welfare of sows and their offspring; and also have practical and financial implications for pig producers. There has been much research into behavioural and physiological changes at parturition (e.g. Lawrence et al., 1994; Jarvis, 1997b), the relationship between piglet mortality and maternal ability (e.g. Andersen et al., 2005), the merits and pitfalls of particular farrowing environments (e.g. Cronin & van Amerongen, 1991) and to a lesser extent, the propensity of individuals to behave in a particular way (e.g. Pitts et al., 2002; Thodberg et al., 2002). Improving production (e.g. by reducing piglet mortality) and/or the welfare of the sows and piglets through genetic selection or environmental manipulation is often cited as the ultimate aim of these studies. An important factor to consider before attempting to breeding for particular characteristics, such as maternal ability, is whether they are related to other aspects of personality.

There is increasing evidence for differences in the behaviour of pigs being attributable to personality traits. Maternal behaviour has many characteristics of a trait, such as a large amount of individual variation and consistency over time (Thodberg et al., 2002; Held, 2006). A fundamental aspect of maternal behaviour is the reaction of sows to their offspring. This is particularly important in first time mothers (gilts), as a small proportion are moderately aggressive towards, and/or savage (infanticide), their piglets. This maternal aggression is heritable, but also seems to be affected by the environment and experience or age (Knap & Merks, 1987; Harris et al., 2003; Jarvis et al., 2004). For example, restriction in farrowing crates might cause frustration by thwarting normal maternal behaviour, including nesting and investigating the first piglets born. This frustration may create an aversive psychological state and impact internal physiological processes, such as the HPA axis, which influences the release of endogenous opioids, as well as hormones such as oxytocin (see Lawrence et al., 1997). This can subsequently affect the progress of parturition and maternal behaviour.

If the degree of stress (and also anxiety and fear) can have negative consequences for maternal behaviour and is more likely in certain personality types, then there should be parallels with other stressful situations. Social confrontation may be one of these situations. Some links have been made between reproduction parameters and aggressiveness or success in social encounters in mice (Mendl & Paul, 1991) and pigs (Mendl et al., 1992; McLean et

al., 1998). Thus it is possible that there are trait-links between aggressiveness at mixing and maternal behaviour. Another commercially relevant behaviour is ease of handling during farrowing: as the stock-person will have more contact with sows in the lead up to, and during the period following parturition than at any other time. Reactivity towards humans may also form part of personality with links to maternal skills and social behaviour (Marchant-Forde, 2002).

This study investigates multiple elements of behavioural style to elucidate whether individual differences in a variety of tests may be attributable to particular personality traits. Various questions were posed relating to whether differences in aggressiveness and success during social interactions translate into differences in reactions to the farrowing environment and maternal ability. These included whether pigs that are more aggressive and/or successful in social confrontations are also more aggressive towards their own piglets and to stockmen. Also, whether there are predictors from behaviour at mixing that might tell us something about behaviour in the run up to and during farrowing? The question of whether the experience of social mixing affects maternal behaviour was also investigated.

The behaviour of 32 gilts was monitored in the eight hours before the birth of their first piglets (referred to as pre-farrowing) and the four hours post onset of farrowing (post-farrowing). This time period was chosen to include the period of high nesting before birth and its subsequent decline. A post-farrowing (onset) phase of four hours was selected as the birth process is often completed within this time and after this point gilts/sows typically show very little activity. Behavioural measures included postures, nest-building activity and gilt-piglet interactions (aggressive and non-aggressive). Gilts were assessed for their reactions towards a stock-person handling their piglets. Saliva was collected for cortisol analysis during pregnancy, immediately before the gilts were moved into farrowing crates, within 24 hours post birth and one week after farrowing. The data were then compared with the measures of mix-aggression and latency to attack in the Resident Intruder Test described in Chapter 3 (RIT4).

5.2 Methods

5.2.1 Animals and housing

Subjects were 36 gilts housed in groups of six. Fours groups were mixed at around puberty (see Chapter 3) and consisted of three pairs of sisters. The other two groups had remained unmixed since weaning and each contained two sets of three sisters. The gilts were treated with altrenogest (Regumate, Janssen Animal Health; 0.4 % suspension, 5 ml daily for 18 days) to synchronise oestrus within groups and artificially inseminated with terminal line semen. Three gilts did not become pregnant and another gilt was unsuitable and was excluded from the analysis.

Gilts were housed in sow pens (detailed in Chapter 3) throughout pregnancy and were moved into farrowing crates between three to five days before the predicted farrowing date. Once in the crates they were continuously monitored for any farrowing problems (e.g. savaging, stuck piglets) via a web camera. Disturbance of the sows during farrowing was avoided to prevent maternal behaviour being altered, but intervention was permitted if a gilt savaged (killed or injured) a piglet. In cases of savaging the piglets were removed, or kept away from the gilt's head for a period of time until she became less reactive. The sows and their litters were kept in standard farrowing crates (1.50 x 3.14 m), with a piglet creep area (0.54 x 2.22 x height 1.05 m, temperature 30 °C). The floor of the creep was covered with wood-shavings and gilts were given a small amount of straw. Each crate had a raised food trough and a nipple water drinker for the sow and a nipple water drinker just above floor level for the piglets. Sows were fed morning and evening with up to 10 kg dry feed (Scotlean Lactating Sow Pellets, ABN).

5.2.2 Behaviour

Continuous video recording (black and white VHS ceiling mounted camera, 24-hour-mode) captured the behaviour of the gilts from entry to the farrowing house. When farrowing appeared imminent (nest-building activity and/or first signs of milky-discharge) a second camera (wide-angle, 12-hour-mode) was mounted on the farrowing crate itself, directed at the head and shoulders of the gilt. This was to ensure that all of the gilt-piglet behaviours could be observed

clearly. Eight hours before the birth of the first piglet and the four hours after farrowing onset were analysed using The Observer (v. 5.0.31, Noldus). Time spent in various postures, frequency of changing between postures and nest-building activity were recorded (Table 5.1). Aggressive gilt-piglet interactions were recorded for the duration of the four-hour observation; non-aggressive interactions were only noted for the first 10 instances of 'piglets-at-the-head'. Piglets were classed as being at the head if they were beyond the front limbs/shoulder and within 30-cm of the ventral side, or 10-cm of the dorsal side of the gilt. If the gilt was lying ventrally then the piglets had to approach within a 30-cm radius of the head. Gilts were classed as savagers if they bit and injured or killed a piglet. Those that performed more than three aggressive behaviours, but without causing injury to the piglet, were classed as moderately aggressive.

Table 5.1 Ethogram of behaviours at farrowing

Category	Behaviour	Description
POSTURES	Stand	Upright with all four feet on ground
	Sit	Front feet and rump on ground
	Kneel	Front legs bent, back feet on ground
	Lying laterally (LL)	Lying on one side, udder exposed
	Lying ventrally (LV)	Lying on udder
	Stepping/walking	Repeatedly moving backwards/forwards
NEST-BUILDING	Bite	Snapping/grabbing at floor or fixtures
	Paw	Scraping motion directed to floor/substrate
	Bar	Rooting (nose) or bashing (head) crate bars
	Root/manipulate	Rooting fixtures/substrate and chewing straw
PIGLET INTERACTION		
Non-Aggressive	Look	Repositions head/directs attention to piglet
	Sniff/nose	Sniff or gentle touch
Aggressive	Aggressive	Bite/attack/violent push/grab (or attempt to)
Other	Raking	Scraping motion with front limbs
	Crush	Sits on piglet

5.2.3 Salivary cortisol

Saliva samples were obtained from the gilts using large cotton buds, which the gilts voluntarily chewed. These were placed in a salivette and spun in a centrifuge at 3000 rpm for five minutes. Sampling was performed in the morning (between 0900 and 1100). The saliva was then decanted into cuvettes, frozen at –28 degrees centigrade and stored until analysis. Samples were taken 37 days before the predicted farrowing date (F-37, two thirds of the way through pregnancy), before moving into farrowing crates (F-3 to F-5), between 12 and 24 hours after birth (F0) and seven days after birth (F7). Farrowing cortisol levels were compared to cortisol levels, in the same pigs, recorded at various points before and after mixing at weaning (Chapter 3) and mixing as gilts (Chapter 4).

Analysis of the samples was performed by standard radioimmunoassay (COAT-A-COUNT® cortisol kit, TKC05, Diagnostic Products Corporation, UK) (Tunn et al., 1992). All samples and duplicates were analysed in the same assay. The detection limit was 0.475 ng ml^{-1} and medium intra-assay coefficient of variation was 18.7 %.

5.2.4 Stock-person directed aggression (SPDA) test

A female stock-person, unfamiliar to the sow, entered the crate and held a piglet for 10 seconds under the belly (causing it to squeal) within 0.5 m of the sow's head. This was repeated with two further piglets. If a piglet did not squeal it was swapped for another. Using the scoring system (Table 5.2) an assessment of the sow's behaviour was made during the process of holding the three piglets, gathering all of the piglets into the creep, weighing, re-marking (days 0, 7, and 14) and giving iron injections (day 2). The test was performed between 0900 and 1100, except for day 0, when it was performed between 12 and 24 hours after birth. For consistency the same two unfamiliar stock-persons were used for all of the tests and the experimenter (present throughout the whole process) and stock-person agreed on the score in each case.

Table 5.2 Scoring system for SPDA test

Score	Behaviour
1	Sow shows no obvious sign of aggression and not disturbed by presence of person.
2	Sow mildly aggressive and may give a few warning vocalisations. May stand or sit.
3	Sow moderately aggressive and gives more warning vocalisations. May attempt to bite if approached. Stands during piglet weighing and directs attention to stock person.
4	Sow very aggressive. Stands quickly and vocalises frequently. Bites crate bars and steps backwards and forwards. Will try to bite if approached.
5	Sow extremely aggressive. Stands rapidly and is extremely vocal. Bites crate bars vigorously and tries hard to escape and/or protect litter. Will readily try to attack person if approached.

5.2.5 Measures of social aggression (from Chapter 3)

Measures of aggression obtained from analyses of behaviour during the gilt-mix (N = 24) were used for comparison with the measures obtained at farrowing. These included: latency to perform an aggressive behaviour; total frequency of aggressive acts; involvement in fights and mutual aggression (fighting and pushing) (frequencies & duration); success in mutual aggression (won, lost and undecided); interrupting fights (frequencies); attacking (total, front & body) (frequencies & duration); short aggressive (SAI) and non-aggressive (SNAI) interactions (frequencies); and received aggression (frequencies). In addition, the summed latency to attack in Resident Intruder Test (RIT) 4 was used as a further measure of social aggressiveness. The success rate (won minus lost/total mutual aggression) in the gilt mix and dominance rank from the group feeding tests (Chapter 3) were used as measures of social status.

5.2.6 Statistical analyses

Pre-farrowing behaviour data were analysed as the summed total for the eight hours prior to farrowing onset, hour by hour and also the total frequency and duration for the four hours pre onset as a direct comparison to the four hours post onset. Post farrowing behaviour data were analysed as the summed total for the four hours recorded. Pre-farrowing postures were analysed individually and as total 'postures'. Pre-farrowing behaviours were categorised as pawing, bars, biting or rooting (Table 5.1) and also totalled (referred to as 'nesting'). The mean and median values quoted in the text, figures and tables were calculated from raw data. Categories were also formed for postures and nesting frequencies based on whether gilts were above (high) or below (low) the median value. Gilts were categorised as aggressive or not aggressive towards their piglets (maternally aggressive) based on whether they performed three or more aggressive piglet-directed acts (push, attack, bite and attempted bite/attack) within the four-hour observation.

The sample size for the analyses varied according to the test being analysed. Of the 36 gilts used in Chapter 4, 32 became pregnant and farrowed. The data for 21 of these gilts were available for comparison with the gilt mix data, as the remaining 11 had not been mixed as gilts. As four gilts were from treatments other than 123 (see Chapter 2), comparisons across farrowing and RIT data were based on 28 pigs.

The majority of the analyses were achieved using non-parametric tests as the data were generally categorical/ranked or not normal. Differences between measures of the same animal (e.g. saliva and SPDA) were analysed using Wilcoxon Matched-Pairs tests. Mann-Whitney U test was used to compare between different categories. Continuous variables were compared for associations using Spearman's Rank Correlation. Categorical data were compared for associations using the Chi-square test. Repeated measures ANOVA (blocking for pig) were used to analyse changes in frequency or duration in pre-farrowing behaviours; non-normal data were transformed (square-root). To compare across all of the different measures taken at various points, the main behaviours, cortisol and test scores were added to a Principle Components Factor Analysis (Varimax rotation, correlational matrix). Parametric (including Factor Analysis) and Chi square statistical analyses were performed using Minitab (v.14); Genstat (v.8) was used for the non-parametric analysis and Repeated Measures ANOVA.

5.3 Results

5.3.1 Pre-farrowing behaviour

Frequency of posture changes and nesting behaviours decreased with time (Figure 5.1). Eight hours before farrowing onset gilts were changing posture approximately once every 90 seconds and performing a nesting behaviour every 86 seconds. This was reduced to once every 2.5 and 4.3 minutes, respectively. High posture change frequency was associated with high nesting frequency behaviour, both one hour before (Chi-Square$_1$ = 4.612, p = 0.032) and over the total eight-hour period (χ_1 = 4.544, p = 0.033).

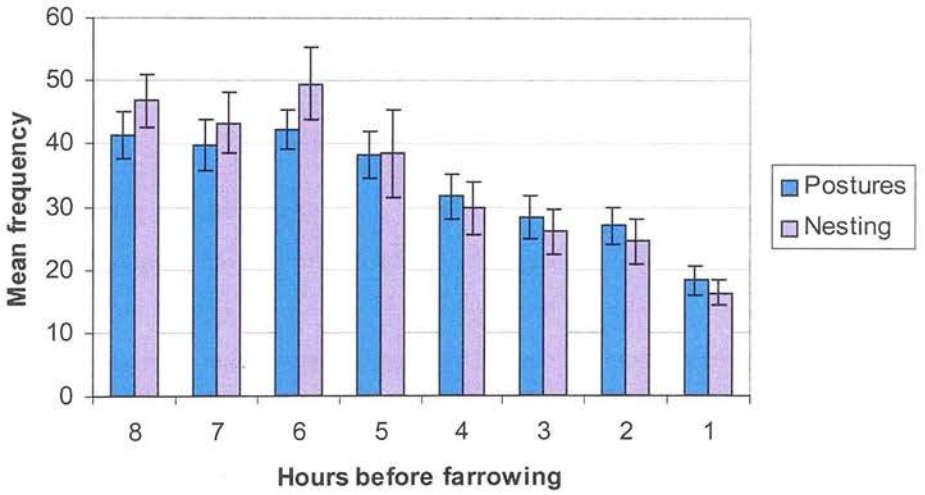


Figure 5.1 Hourly frequency of posture changes and nesting behaviour during the 8-hour period before farrowing (Repeated measures ANOVA, posture change, variance ratio = 13.85, p < 0.001; nesting variance ratio = 10.52, p < 0.001)

5.3.1.1 Postures

Standing, kneeling and stepping (frequencies and duration), showed a clear time effect (Repeated Measures ANOVA$_{7, 231}$, p < 0.001), with all three behaviours declining as farrowing approached (Figure 5.2). Sitting increased in duration and frequency (p < 0.001). Lying laterally (LL) and ventrally (LV) were the most frequent postures. There was a trend for a decrease in the frequency of LL (p = 0.086) with the duration spent in this behaviour increasing (p < 0.001). The frequency of LV peaked four to five hours before farrowing (p = 0.043), although the duration spent in this posture did not change significantly.

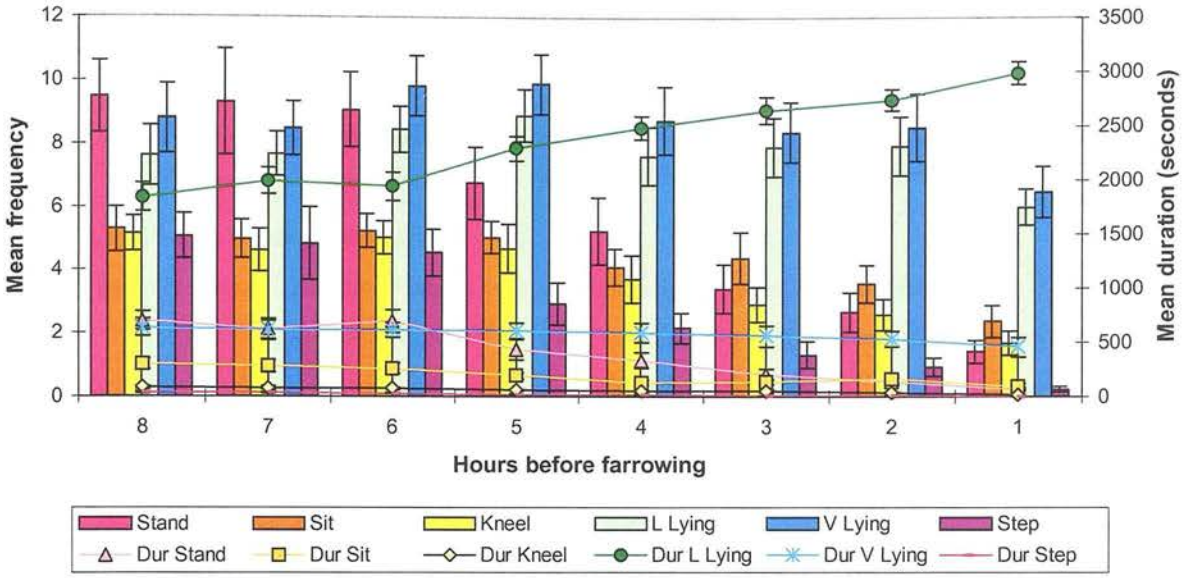


Figure 5.2 Mean posture frequency (bars) and duration (lines) each hour in the 8 hours before farrowing

5.3.1.2 Nesting behaviours

The most frequent behaviour was rooting, which decreased over time (Repeated Measures $ANOVA_{7,231}$, $p < 0.001$), as did bar ($p < 0.001$) and biting behaviours ($p = 0.002$). In most pigs biting behaviour had stopped altogether by one to two hours before farrowing. Pawing the ground however increased as farrowing approached ($p < 0.001$) (Figure 5.3).

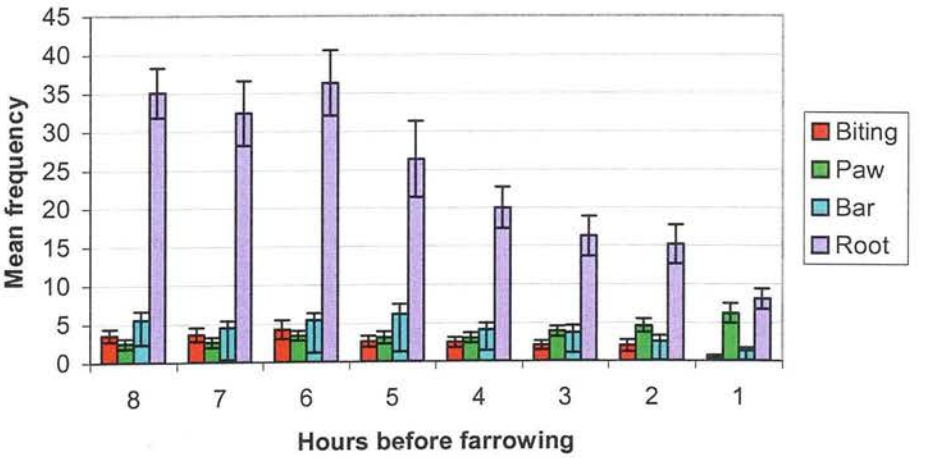


Figure 5.3 Frequency of nesting behaviours in the eight-hour period before farrowing

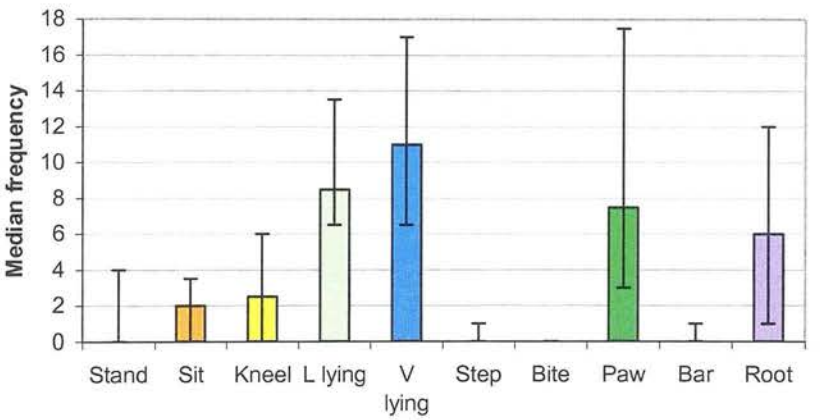
Biting and bar were positively correlated (Spearmans$_{30}$ = 0.639, p = 0.003) and to a lesser extent, rooting and pawing were associated (r_s = 0.451, p = 0.052).

5.3.2 Post-farrowing behaviour

5.3.2.1 Postures and nesting

The frequency of posture changes was substantially lower in the four hours after farrowing, compared to the four hours before farrowing (median pre = 90.5, post = 29.5, Wilcoxon$_{31}$ = -13.0, p < 0.001). During the four hours after farrowing onset LV and LL were the most frequent postures (Figure 5.4a); stepping and standing were uncommon. Gilts spent very little time in postures other than LL (Figure 5.4b).

(a)



(b)

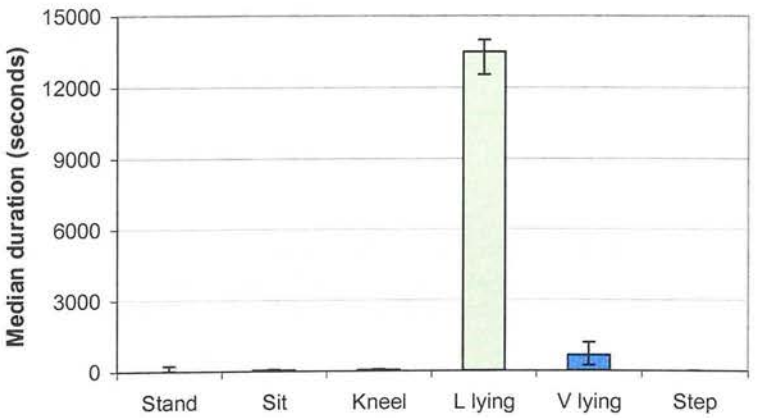


Figure 5.4 Frequency (a) and duration (b) of postures and nesting behaviours in the four-hour period after farrowing (with interquartile range)

Some nesting behaviour persisted once farrowing had begun (Figure 5.4a). The frequencies of rooting ($U = 54$, $p < 0.001$), biting ($U = 118.0$, $p < 0.001$), and bar behaviour ($U = 111.0$, $p < 0.001$) were greatly reduced in the four hours after the onset of birth, compared to the four hours before. There was a non-significant decrease in pawing (median pre = 13.5, post = 7.5).

5.3.2.2 Piglet-directed behaviour

Most piglet-directed behaviour involved sniffing/looking (Figure 5.5), very few pigs acted aggressively. Of the 32 gilts, two were considered to be 'savagers' and intervention was required at the birth to prevent further injury to piglets. Another gilt was very reactive and directed 42 aggressive acts to piglets (mostly violent pushes) within the observation period. The other six gilts classed as aggressive to piglets or 'maternally aggressive' (MA) performed between three and six aggressive acts. Raking was not considered to be an aggressive act as it was rarely performed with such force as to harm piglets; it looked similar to and was correlated with post-farrowing pawing behaviour ($r_s = 0.416$, $p = 0.025$).

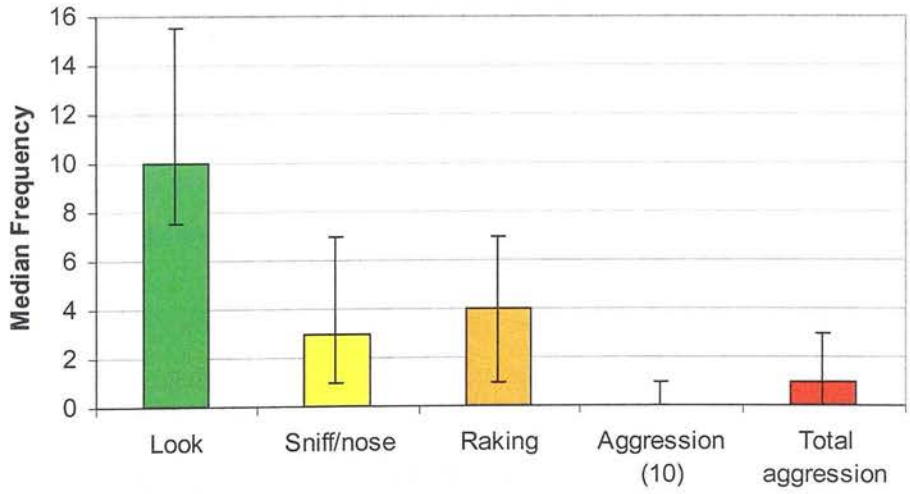


Figure 5.5 Frequency of piglet-directed behaviours: look, sniff, raking, and aggression (10), for the first 10 periods of 'piglets at head'; also total aggressive behaviours recorded (total aggression) within the four-hour observation period (with interquartile range)

Piglet-directed aggression was not associated with high/low categories of posture changes or nesting behaviour pre-farrowing. Post-farrowing, MA gilts changed position more frequently

(median, MA = 34.5, non-MA = 24.5, Mann-Whitney$_{21}$ U = 15.0, p = 0.01), had higher frequencies of LL (MA = 14.0, non-MA = 7.5, U = 24.5, p = 0.069), LV (MA = 17.0, non-MA = 9.5, U = 17.5 p = 0.017) and sitting (MA = 1.5, non-MA = 4.0, U = 23.0, p = 0.054).

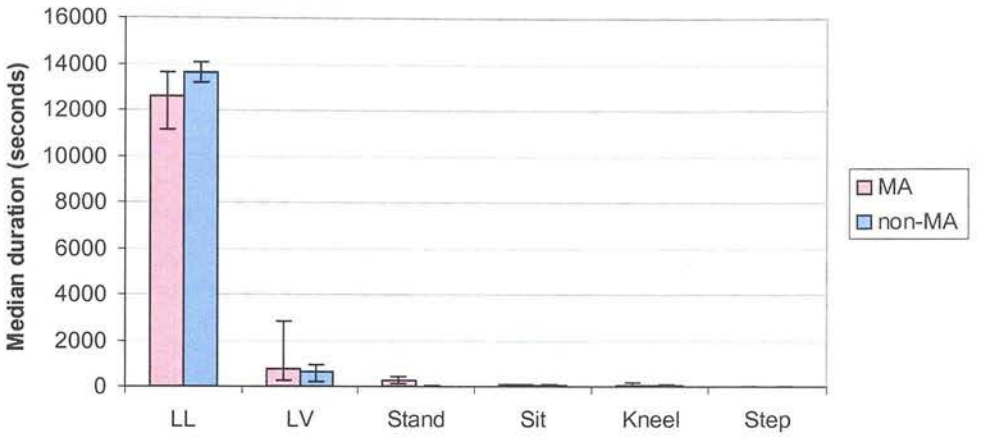


Figure 5.6 Duration of post farrowing postures for maternally aggressive (MA) and non-aggressive gilts (with interquartile range)

During the 4 hours after farrowing, non-MA gilts spent approximately 15 minutes longer than MA gilts LL (U = 23.0, p = 0.012). MA gilts spent more time LV (U = 23.0, p = 0.025), standing (U = 23.0, p = 0.027) and stepping (U = 23.0, p = 0.028) than non-MA gilts (Figure 5.6). The MA gilts tended to be more responsive to piglets, directing more non-aggressive behaviours (look, sniff and nose) towards them (median frequency, non-MA = 11.0, MA = 22.0, U = 59.5, p = 0.065). There was no difference in overall nesting behaviour post farrowing onset. Of the individual nesting behaviours there was a tendency for an effect of MA on rooting (median non-MA = 4.0, MA = 10.0, U = 24.0, p = 0.063).

5.3.3 Stock-person directed aggression (SPDA)

None of the gilts were considered as excessively aggressive towards the stock-person as none scored more than 3 at any point. The scores were lowest on day 14 (day 14 & 2, Wilcoxon$_{15}$ = 22.5, p = 0.03; 14 & 7, W$_{10}$ = 5.0, p = 0.02) with day 0 being intermediate between day 14 and days 2 and 7 (Figure 5.7).

SPDA was not related to MA or postures pre/post-farrowing. It was associated with continued nest-building behaviours after the onset of farrowing (r_{s27} = 0.572, p = 0.001). Of the piglet-directed behaviours, SPDA was correlated to raking piglets (r_{s30} = 0.508, p = 0.003).

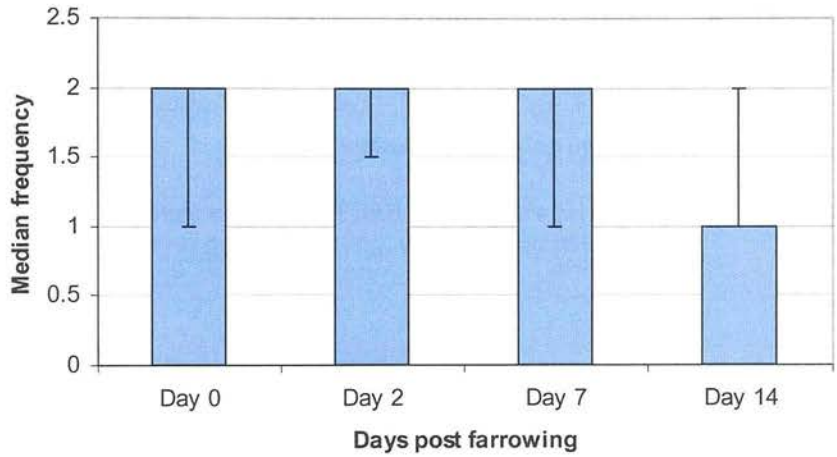


Figure 5.7 Median score for stock-person directed aggression (SPDA), with interquartile range, on the day of farrowing and days 2, 7 and 14 after farrowing

5.3.4 Salivary cortisol

Salivary cortisol on day 5 before farrowing, taken prior to the gilts being moved into the farrowing house, was significantly higher than at all of the other points (F-37, $Wilcoxon_{32}$ = -37.0, p < 0.001; Farrow, W = 55.0, p < 0.001; F+3, W = 19.0, p < 0.001; F+7, W = 17.0, p < 0.001) (Figure 5.8). Cortisol within 24 hours of farrowing was higher than three and seven days after farrowing (F+3, W = 134.5, p = 0.014; F+7, W = 160.0, p = 0.052).

Low levels of cortisol 37 days before farrowing were associated with high levels of rooting pre-farrowing ($Spearmans_{19}$ = -0.604, p = 0.006). There was a weak tendency for MA gilts to have higher cortisol at F–37 (medians, MA = 2.6, non-MA = 1.8, U = 65.0, p = 0.1). Although salivary cortisol for MA gilts was lower at F-3/5 days, this was not significant (MA = 5.0, non-MA = 8.3).

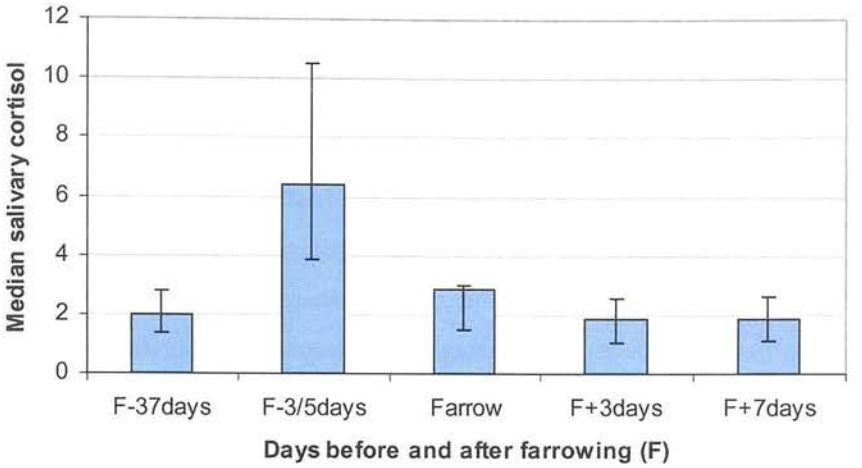


Figure 5.8 Salivary cortisol (37 and 5 days) pre farrowing, within 24 hours of farrowing and (3 and 7 days) post farrowing, with interquartile range

5.3.5 Mixed versus non-mixed

There was a difference in nesting behaviour frequency during the eight hours before farrowing, with 62 % of mixed pigs and 18 % of non-mixed gilts showing higher than median nesting behaviour (Chi-square$_1$ = 5.895, p = 0.015). Looking at the nesting behaviours separately this relationship was true for pawing (Mann-Whitney$_{21,11}$ = 67.0, p = 0.054) and rooting (U = 58.5, p = 0.023) but not biting and bar. There was no difference in postures before farrowing or aggression towards piglets after farrowing according to whether gilts were mixed at puberty.

5.3.6 Comparison to measures from the Gilt Mix (Chapter 3)

5.3.6.1 Pre-farrowing

Pigs that changed postures most frequently in the eight hours before farrowing were faster to attack in RIT4 (median attack latency, high posture change = 309.0 seconds, low = 340.0, U_{21} = 69.5, p = 0.028) and were involved in less fights with an undecided outcome in the gilt mix (high = 0, low = 1.5, U = 26.0, p = 0.034). Pigs performing more nesting were slower to attack in RIT4 (high nesting = 380.0 seconds, low = 280.0 s, U = 24.0, p = 0.045) and involved in more indecisive fights (high = 0, low = 2.0, U = 12.5, p = 0.002). They were also involved in less mutual aggression (high = 3.0, low = 7.5, U = 25.0, p = 0.058) and were slower to perform an aggressive behaviour (high = 72.36 s, low = 31.74 s, U = 21.0, p =

0.023) during the mix. High nesting gilts spent less time attacking (high = 54.24 s, low = 109.3 s, U = 23.0, p = 0.037) and tended to direct fewer attacks to the body of other pigs (high = 2.0, low = 7.5, U = 25.5, p = 0.054).

Specific types of nesting behaviour (bar, paw, root and biting) were associated with different mix behaviours. Bar behaviour was negatively associated with aggression in the mix, including frequency (Spearmans = -0.536, p = 0.018) and duration of mutual aggression (r_s = -0.540, p = 0.017), frequency of total aggression (r_s = -0.522, p = 0.022), and undecided fights (r_s = -0.478, p = 0.038). There were tendencies for more bar behaviour to be associated with fewer occurrences of fighting (r_s = -0.440, p = 0.059) and less time attacking (r_s = -0.436, p = 0.062). More frequent biting behaviour in gilts was associated with receiving less aggression (r_s = -0.494, p = 0.032), directing fewer attacks to the front (r_s = -0.682, p = 0.001) and body (r_s = -0.506, p = 0.027) and losing fewer fights (r_s = -0.479, p = 0.038). Rooting was strongly correlated with taking longer to perform an aggressive behaviour in the mix (r_s = 0.809, p < 0.001).

5.3.6.2 Post-farrowing

Nesting behaviour was less frequent in pigs that were interrupted more frequently during bouts of aggression (r_s = -0.616, p = 0.003), as well as those that directed more attacks to the front of others (r_s = -0.562, p = 0.014) and spent longer attacking in this position (r_s = -0.469, p = 0.032). Posture change frequency post birth onset did not correlate with any of the mix behaviours. Gilts that were aggressive towards their piglets attacked more frequently (median frequency attacks, high MA = 11.0, low MA = 5.0, $U_{14,7}$ = 23.0 p = 0.055), performed more SAI (high MA = 15.0, low MA = 9.5, $U_{14,7}$ = 20.0 p = 0.029) and aggression overall (high MA = 38.0, low MA = 18.5, $U_{14,7}$ = 21.0 p = 0.038), in the gilt mix. They also tended to win more fights (high MA = 5.0, low MA = 1.0, U_{21} = 24.0, p = 0.06).

5.3.6.3 Salivary cortisol

Cortisol levels five days before farrowing were higher than at any other point measured (Wilcoxon, p values from 0.002 to < 0.001) (Figure 5.9). The second highest point, within 24 hours of farrowing, was significantly higher than 30 minutes after mixing at weaning (W = 50.0, p = 0.022), 7 days after the weaning mix (W = 21.0, p = 0.016), the gilt mix baseline (W = 23.0, p < 0.001) and cortisol levels 7 days post mixing as gilts (W = 5.0, p < 0.001).

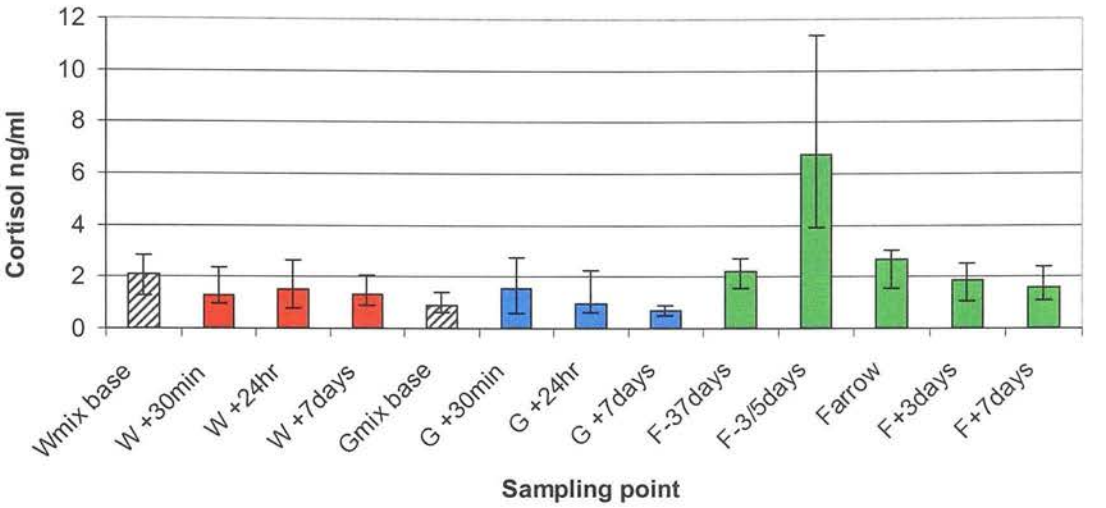


Figure 5.9 Salivary cortisol levels (median and interquartile range) in female pigs before and after mixing at weaning (W), mixing as gilts (G), and farrowing (F) (data for gilts tested at all points, n = 21)

5.3.7 Factor analysis

The four main factors extracted explained 64.6 % of the variation (Table 5.3). All axes had behaviours loading strongly (loading of > 0.5) at both extremes, with the exception of factor 2, where the greatest loading describing the negative dimension was received aggression (-0.332). The first two factors explained 41.2 % of the variation and were dominated by behaviours relating to aggression in the mix, pre-farrowing behaviour, cortisol 37 days pre-farrowing and piglet-directed aggression (Figure 5.10).

Table 5.3 Factor loading values for the main behaviours extracted from pre-farrowing nesting and posture change frequency, post-farrowing piglet and stock-person interactions, cortisol and measures from the gilt mix and summed attack latency (AL) in resident intruder test 4 (RIT). The parameters (+, pink; - blue) loading most strongly are highlighted for each axis

VARIABLE	Factor 1	Factor 2	Factor 3	Factor 4
Pre-farrowing				
Root	**0.695**	-0.216	-0.169	-0.154
Bar	**0.816**	-0.176	-0.203	0.169
Paw	0.414	0.330	**-0.716**	-0.187
Biting	**0.732**	0.166	-0.021	0.124
Posture change	**0.806**	-0.080	-0.144	-0.115
Post-farrowing				
Total look/sniff	0.224	0.372	**0.710**	0.024
Total piglet-aggression	-0.105	**0.463**	0.021	-0.171
Mean SPDA	-0.271	0.134	0.242	**-0.625**
Cortisol				
F-37	**-0.472**	0.279	-0.036	0.364
F-3 to -5	-0.243	-0.002	0.134	**0.788**
F+1	0.213	-0.202	**-0.644**	0.191
Gilt Mix				
Attack	-0.003	**0.790**	0.257	0.306
Mutual Aggression	-0.406	**0.777**	-0.161	0.051
Received Aggression	**-0.468**	**-0.332**	-0.300	**-0.513**
Undecided fights	**-0.750**	0.253	-0.388	0.177
Fights won	0.025	**0.879**	0.248	0.131
RIT				
Attack Latency RIT 4	0.306	**-0.321**	0.137	**-0.491**
Variation Explained (Total 64.6%)	**23.6%**	**17.6%**	**11.8%**	**11.6%**

The matrix indicates that a low frequency of pre-farrowing behaviours was correlated with receiving a lot of aggression in the mix and having higher cortisol in the last trimester of pregnancy (and three days before birth). Pigs performing more aggressive behaviour and winning more fights during the mix received less aggression and were more aggressive towards piglets.

Using the behaviours loading most strongly on the positive and negative extremes of each factor axes the following descriptors were formed:

- Factor 1 = Pre-farrowing activity – high cortisol in late pregnancy & more frequent received aggression and undecided fights in the mix
- Factor 2 = Attacking, fighting and winning fights & piglet-directed aggression – more aggression received in the mix & longer attack latency in RIT4
- Factor 3 = Looking/sniffing piglets – high pre-farrowing pawing & post-farrowing cortisol
- Factor 4 = High cortisol before farrowing – higher SPDA & received aggression during mixing

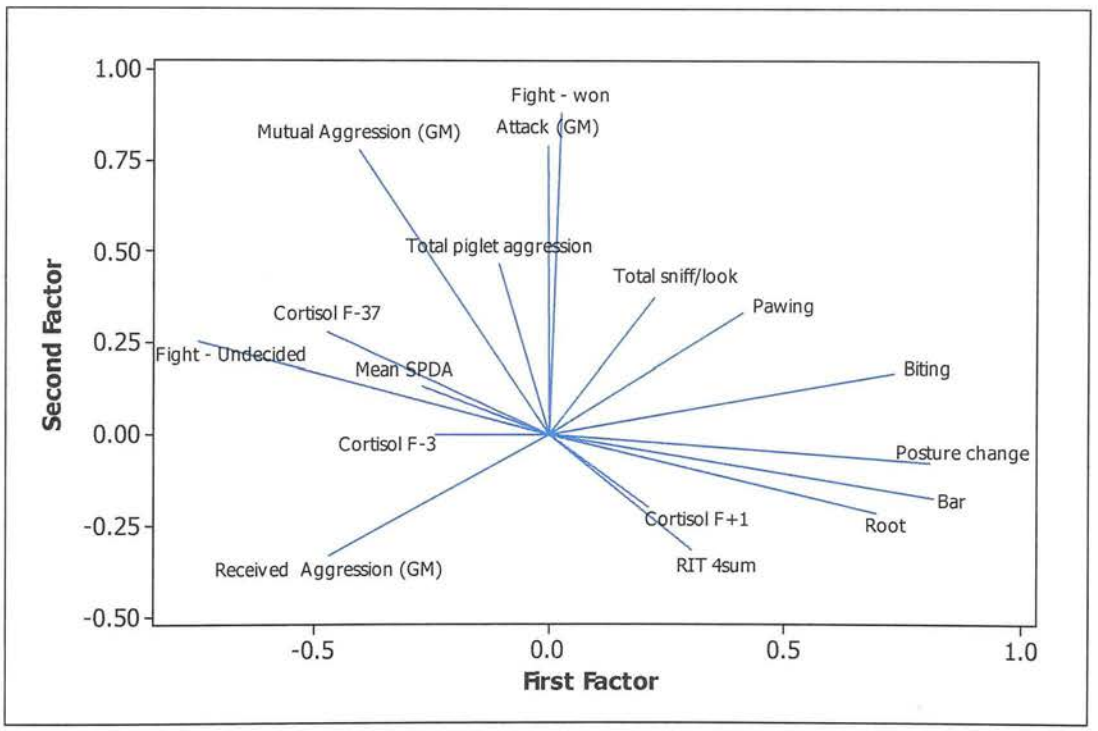


Figure 5.10 Loading plot of behaviours explaining factor dimensions 1 (pre-farrowing activity – high cortisol in late pregnancy & more frequent received aggression and undecided fights in the mix) and 2 (Attacking, fighting and winning fights – more aggression received in the mix & longer attack latency in RIT4)

5.4 Discussion

5.4.1 Pre-farrowing behaviour

The eight hours pre-farrowing were characterised by a period of high activity, with gilts changing posture and performing nesting-type behaviours frequently. Activity was greatly reduced immediately before the onset of farrowing; sitting and the lying postures increased and as would be expected, standing, kneeling and stepping declined. The nesting behaviours generally showed the same pattern of initially high activity with a gradual progression towards immobility. Rooting was the most frequent nesting behaviour, although as with biting and bar behaviour, frequencies declined considerably before farrowing. This high frequency of posture changes and nesting behaviour and subsequent decrease in activity was typical of pre-farrowing behaviour in crated gilts (Lawrence et al., 1994; Jarvis, 1997a; Jarvis et al., 2002).

The pre-farrowing nesting behaviour of the gilts showed some parallels to that reported in free-ranging sows (see Jensen, 1986; Jensen, 1993). In natural conditions the sow investigates potential nesting sites 15-24 hours before parturition. Once an appropriate site is selected the sow digs a hole, which is then lined with soft material using scrapping movements of the front hooves (pawing) and is arranged using the snout (rooting). The sow also uses her mouth to collect and add more material, including harder materials such as branches. Once completed the sow will lie in the nest and may perform more nesting behaviour before the onset of farrowing. In this study gilts rooted and pawed the floor and crate fixtures and although these substrates were very different from natural conditions, the behaviours would seem analogous. Additionally, rooting and pawing were associated with each other, which has also been reported in natural conditions (Jensen, 1993; Jensen et al., 1993). While lining the nest the sow may make 'nodding' movements to spread nesting material (Jensen, 1986). Whilst this was not specifically recorded (as gilts were typically given very little straw to manipulate) some of the crated gilts were observed to toss the straw in the air. Biting and bar-directed (bashing/rooting bars) behaviours were associated. It is less clear whether these are analogous to specific nesting behaviours, or whether they are indicative of frustration from restraint. Biting and bar behaviour peaked 5-6 hours before farrowing started, which would be consistent with the period of collecting and arranging nest materials (Gustafsson et al., 1999). Furthermore, biting of pen fixtures occurs in unrestrained gilts given nesting material (Jensen, 1993; Lawrence et al., 1994; Jarvis et al., 2001). The

behaviour may persist in these situations as the nesting material supplied (pre-cut straw) might provide insufficient feedback in terms of the properties of the material, or the effort required to collect it.

Unlike the other nesting behaviours, pawing the ground actually increased as farrowing approached and was greater in the four hours after farrowing compared to the four hours prior to farrowing. Jensen (1993) suggested that pawing remains elevated for most of the nesting period as it occurs both in the initial nesting phase to create a hole and scratch nest material into the nest, but also in latter nesting stages to re-arranging the nest. Nesting is usually considered to cease at (or very shortly after) the onset of birth (Petersen, 1990), although continued nest-building after the onset of birth in not unheard of (Damm et al., 2003; Jarvis et al., 2004). The gilts in this study may have continued pawing as a result of continued motivation to nest arising from inadequate provision of suitable materials, as the provision of nesting material has been shown to reduce the amount of nesting performed after the onset of farrowing (Thodberg et al., 1999). Pawing may also have been an indicator of discomfort or contraction pains, with the increase in frequency signalling the beginning of the birth process. This link has not been reported in the literature, although movement of the upper hind limb has been linked to contraction pain (Petersen, 1990).

5.4.2 Post-farrowing behaviour

As in other studies of post-farrowing behaviour, the gilts tended to direct attention (sniff/look) towards the first piglets born and then lie in lateral recumbency for several hours (Jensen, 1986; Petersen, 1990; Harris & Gonyou, 1998; Jarvis et al., 2004). The pigs were recorded as changing position a number of times within the four-hour observation period, albeit at a vastly reduced rate to the four hours prior to farrowing onset. This unresponsiveness may be the result of a number of physiological processes, such as opioid-mediated passivity (Jarvis et al., 1999). The lack of movement once the birth process has begun is likely to have numerous benefits for the piglet, including reducing the chance of crushing, increasing opportunities to suckle and aiding thermoregulation. As farrowing in domestic pigs can typically last between 30 minutes and six hours (van Rens & van der Lende, 2004) minimising unnecessary movement will also conserve the sow's energy.

5.4.3 Maternal aggression

Most gilt-piglet interactions did not involve aggression (sniff/look/nose). Maternal-aggression was apparent in approximately 28 % of gilts: two gilts attacked piglets and another performed behaviours that could have directly caused piglet deaths (9.4 %); six others performed a few (between three and six) clearly aggressive behaviours (18.8 %) during the four-hour observation, but generally posed no significant danger to their piglets. The six mildly aggressive gilts could not be considered as savagers, but like the three very MA gilts they were more 'reactive' during farrowing and did not show the typically inactive behaviour of the non-aggressive gilts. These figures are comparable with other recorded incidences of maternal aggression in first parity domestic and wild-boar sows of between 21 % and 33 % (McLean et al., 1998; Harris et al., 2001; Ahlstrom et al., 2002; van Rens & van der Lende, 2004). There was no clear link between pre-farrowing behaviours and subsequent maternal aggression, although it could be that the sample size here was too small to detect any precursor attributes of MA. As demonstrated elsewhere, maternally aggressive sows were more active after the onset of farrowing than non-aggressive sows and were more responsive to piglets (Harris et al., 2001; Ahlstrom et al., 2002; Jarvis et al., 2004).

In many studies either no distinction is made between moderate aggression and savaging (infanticide), or no precise definition is given. Where a distinction has been made it appears that, as in this study, savaging occurs at lower rates to moderate aggression and is generally between 7 % and 10.5 % (Harris et al., 2001; Ahlstrom et al., 2002; Marchant Forde, 2002; van Rens & van der Lende, 2004). In commercial practice savaging is considered a minor cause of mortality compared to crushing, stillbirths and low viability piglets (Edwards, 2002) and larger-scale studies tend to find maternal aggression at lower rates. Knap & Merks (1987) for example, found 8 % of 1477 gilts were aggressive, although there was some variation by breed (7.4 % to 13 %). In a large-scale study of seven commercial farms (7407 gilts and sows) 3.4 % of gilts savaged (Harris et al., 2003). One reason for the difference in commercial conditions could be that moderate aggression is less likely to be picked up (Harris et al., 2003). It is likely that there will also be less human-interference during farrowing. When studying farrowing there is a trade-off between gathering information and disturbing gilts and this is a particular problem in maternally aggressive gilts, as they tend to be more reactive. The presence of people could potentially increase the reactivity of otherwise unreactive gilts, or cause moderately aggressive sows to become savagers. This theory is unproven, although two studies with particularly high-levels of piglet-directed

aggression also had high levels of human intervention during farrowing: in the study by McLean et al. (1998) 24 % of gilts required treatment with sedatives; Jarvis et al. (2004) classed 41 % of crated gilts classed as savagers. In both studies, people were present in the room throughout farrowing, and sows were regularly blood sampled (via a catheter). In McLean et al. (1998), piglets were removed, weighed, and kept away from the sow until two hours after placental expulsion, a process which is likely to have caused the piglets to squeal. The influence of human presence should not be underestimated (see Hemsworth, 2003). It is conceivable that people being present at farrowing may heighten emotional responses, such as stress, fear and anxiety, which are factors that have been linked to savaging (Jarvis et al., 1999; Jarvis et al., 2006) piglet mortality (Hemsworth et al., 1999; Janczak et al., 2003b) and sow responsiveness to piglets (Pedersen et al., 2003). Because of the relatively low incidence of savaging it is difficult to distinguish whether true savagers were motivationally different from the moderately aggressive sows. This is an important consideration when examining piglet-directed aggression as a part of personality, as aggression by a dominant/aggressive pig would be functionally different from gilts savaging out of fear, anxiety or a psychological disorder. As there is currently little evidence to suggest that this is the case, the moderately and severely aggressive gilts in this study were treated as if they were on the same scale of maternal aggressiveness with savaging at one extreme.

5.4.4 Stock-Person Directed Aggression (SPDA)

Gilts might be expected to be more reactive when the piglets are newborn and at their most vulnerable. In rats for example, the females become more aggressive when they have pups to protect, which is thought to be a result of reduced fear (Hansen & Ferreira, 1986). However as already mentioned, for a period of several hours post-farrowing they generally appeared to be unresponsive, and as a consequence SPDA scores on day 0 were intermediate between day 14 and days 2 and 7. SPDA had declined by day 14, which would be expected. In natural conditions the nest area is abandoned after about 7-10 days and the sow and her litter join the social group (Jensen, 1986). Although piglets still suckle and remain close to the sow, they will be more robust and increasingly independent. Despite SPDA scores not being directly related to maternal aggression, those gilts more reactive to people were possibly more reactive after farrowing onset, as they continued to paw the ground and more frequently raked piglets.

None of the gilts were considered as excessively aggressive towards the stock-person in the two-week period following farrowing. High levels of SPDA (including the highest category) were observed in the farm-sows housed in the same conditions. Some of the MA pigs appeared capable of being aggressive towards the stock-person in their heightened state of reactivity during farrowing, but by the first testing point none of the gilts scored more than moderate aggression (score 3 – standing and vocalising). The relatively low levels of SPDA could have been a consequence of the sample size (N = 32), although Marchant Forde (2002) reported similar levels for 62 first parity gilts (mean score 1.33). As high levels of SPDA are undesirable from the perspective of the stock-person, it may be that selective breeding has reduced its occurrence and the relatively low levels might be representative of domestic sows. An alternative explanation is that the experimental gilts had received human contact on a daily basis from birth and were generally unafraid of the experimenter. The gilts may have generalised their passive attitude towards the experimenter to the unfamiliar stock-person, particularly as both were female; using a male stock-person may have yielded different results. Positive handling can have beneficial effects on subsequent human-interactions by reducing fear of humans (Andersen et al., 2006; Hemsworth & Barnett, 1992). The farm sows had received much less human contact during their life and the attention they received (e.g. mucking out) could not generally be considered positive (or negative). In a test similar to the one used in this study, Held (2006) found reactions declined within and between parities, which could be attributable to numerous factors, including a reduction in fear. Contrary to this, gilts classed as 'bold' in a pre-farrowing human approach test scored higher in SPDA tests, indicating that it was the confident pigs that showed more SPDA (Marchant-Forde, 2002). Janczak et al. (2003b) proposed that two separate dimensions of personality, fear of humans and anxiety from a novel environment, are related to different aspects of maternal ability. Thus it would be possible for high-anxiety gilts to have a lack of fear of people (leading to higher SPDA score); this might account for the lack of a direct link between reactivity in the SPDA test and reactivity towards piglets. Spinka et al. (2000) mentions the characteristics 'calmness' and 'protectiveness' (which could be analogous to anxiety and fearfulness respectively) as important dimensions in describing variability in maternal traits. Further analysis would be required to elucidate whether the gilts in this study were responding according to anxiety from the environment (e.g. maternal aggression) or confidence towards people.

5.4.5 Salivary cortisol

Cortisol levels were greatest in gilts three to five days pre-farrowing (before being moved into farrowing crates), followed by 12-24 hours after farrowing. Levels were greater than at any other point throughout the entire study, including mixing into new social groups. Cortisol has been found to increase, in response to being moved into farrowing accommodation, possibly as a result of confinement (Jarvis et al., 2002); within 24 hours of farrowing, linked to nesting behaviour; and during physiological processes in preparation for birth (Jarvis, 1997a; Bazer et al., 2001; Jarvis et al., 2002), and around parturition itself (Lawrence et al., 1994; Jarvis et al., 1998). Cortisol then drops to pre-farrowing levels by the day after farrowing. A rise in cortisol similar to the one presented here, several days before farrowing, has not previously been reported. The sampling point was estimated at three to five days before predicted farrowing but the actual number of days varied between 0.5 and 5.5 days (mean ± SD = 2.73 ± 1.1 days). As only two gilts farrowed within 24 hours of sampling it seems unlikely to have biased the cortisol result and there was nothing statistically to suggest that there was an interaction between cortisol level and the difference between gilts in the number of days before farrowing. Salivary cortisol across the experiment was comparable to levels reported elsewhere (Ekkel et al., 1997; Geverink et al., 2002) and the peak just before farrowing was comparable with levels seen after challenge with ACTH (Ruis et al., 2000). In pregnant women, not only is cortisol higher in late compared to early pregnancy, but the effects of stressors are also greater, causing larger increases in cortisol (Obel et al., 2005). The peak three to five days before farrowing could have been a response to an environmental stressor, the effect of which was exaggerated due to imminent farrowing. Considering any other challenges that may have been impacting on the physiology of gilts, they were feed restricted, which has been shown to cause frustration (e.g. Appleby & Lawrence, 1987) and presumably at this late stage in pregnancy their metabolic demand was greatest. In a study of free-ranging pre-parturient sows the first behavioural changes occurred two to three days before farrowing (Jensen, 1986). At this point sows left the social group to investigate potential nesting sites and some even constructed 'mock nests'. The rise in cortisol before farrowing could therefore have been a response to thwarted exploratory behaviour and the inability of sows to isolate themselves from group-mates.

There were some connections between cortisol and behaviour pre and post-farrowing. Gilts with higher cortisol levels at the start of the third trimester (37 days prior to farrowing) performed less frequent rooting behaviour pre-farrowing; it also appears that these gilts may

have been more likely to be aggressive to their piglets. Maternal cortisol levels immediately post partum have been correlated with maternal grooming, but not maternal aggression, in sheep (Dwyer et al., 2004). There is an indirect link between maternal ability and cortisol in pigs: crated gilts have higher cortisol levels before and during farrowing, as opposed to those in farrowing pens, and have been shown to differ in maternal ability (Lawrence et al., 1994; Jarvis et al., 2002). In the initial period post-birth-onset crated gilts are more active and responsive to piglets (Jarvis et al., 2004) and may even be more likely to show maternal aggression (Jarvis et al., 2004). The differences in pre-farrowing nesting behaviour and activity in theses systems is presumably due to increased restriction, and often a lack of nesting substrate in crates (Jarvis, 1997a; Jarvis et al., 2001; Jarvis et al., 2002). Although all of the gilts in the present study were housed in the same conditions (crates), some were more reactive during and after farrowing than others; differences in this reactivity may have been evident in cortisol levels over 30 days prior to farrowing.

5.4.6 Comparison to aggression during the gilt mix and RIT4

Pre-farrowing and post-farrowing behaviours were linked to aggression during the gilt-mix and RIT. Frequent posture changes pre-farrowing were made by pigs that were faster to attack in RIT4 and that were involved in more decisive fights in the gilt mix. More frequent nesting appeared to be performed by less aggressive gilts, as they had been involved in less mutual aggression, spent less time directing attacks to the body of other pigs and were slower to attack in both RIT4 and the mix. Specific types of nesting behaviour (bar, root and biting) appeared to be associated with different types of mix aggression, with associations generally in the direction of less mix-aggression. Pigs mixed as gilts showed higher levels of nesting than the non-mixed gilts, particularly pawing and rooting. There did appear to be a connection between being mixed as gilts and maternal aggression (33.3 % mixed maternally aggressive, 18.2 % unmixed), but this was not significant. A larger sample size would probably be needed to explore this relationship. Nevertheless, gilts that were aggressive and successful in the mix showed higher levels of maternal aggression.

Few other studies have found connections between social aggression and maternal behaviour in pigs. McLean et al. (1998) did not find a link between success in aggressive encounters and maternal aggression, although they did propose that maternally aggressive gilts were involved in less social aggression. These results conflict with our findings. The reasons for this could be a consequence of differences in the definitions and measurements of mixing-

aggression and maternal aggression. The ethogram used in McLean et al. (1998) was much less detailed than here and as mentioned previously, there was an unusually large number of 'savagers' reported. Csermely (1991) used a feed competition test to assign dominance and found that the dominant animals were more active pre-farrowing. Whilst we found no connection between dominance in food competition tests and farrowing behaviour, frequent posture changes were associated with aggression, and fights with a clear winner.

5.4.7 Conclusions

Farrowing in gilts and sows can be defined by a series of distinct behavioural and physiological changes. Whilst these changes tend to follow a pattern defined here and elsewhere in the literature, particular phases can show a great deal of variation between individuals. Different aspects of maternal behaviour appear to be inter-linked, such as maternal aggression and restlessness post-farrowing. These behavioural responses may be linked to emotional states such as levels of anxiety or fear. Furthermore, differences in maternal behaviour have been shown in this study to be related to aggressive behaviour in social interactions before pregnancy. It has been demonstrated that there are individual differences between pigs, that these differences show some stability over time (previous Chapters) and across situations (correlates between social behaviour and maternal behaviour). So to conclude, it appears that personality traits influence maternal and social behaviour, although responses are not hard-wired and will be modulated by experience, current environment and internal physiological state.

CHAPTER 6

GENERAL DISCUSSION

The overall aim of this thesis was to demonstrate whether aggressiveness is a trait of individual pigs, and to understand aggression in a wider context, including social success, physiological stress responses and behaviours that may not obviously seem related to aggression (e.g. play, maternal behaviour). Mixing together unfamiliar pigs is a routine management practice in indoor-reared pigs, which often results in excessive fighting and aggression. This ferocity of aggression in artificial conditions is not seen in wild or free-ranging pigs and may be motivated by the desire to form a social hierarchy or to drive away the unfamiliar animals (Rushen, 1988). Individual pigs differ in the quantity and severity of aggression displayed in these circumstances, which could be due to differences in resource holding potential (e.g. weight, experience, success), or be the result of an aggressive trait. There is evidence for trait aggression: with definable differences between pigs (Erhard et al., 1997); that are consistent within individuals and situations (Erhard & Mendl, 1997; D'Eath, 2004); and show some repeatability over motivationally similar situations (e.g. all types of social conflict) (D'Eath, 2002). Although, (to my knowledge) aggressiveness over lifetime (from birth to post-puberty) has not previously been reported. If trait aggression is a part of the personality profile of pigs then it should be related to other behavioural or physiological characteristics and be predictable across dissimilar situations. Studies on this basis have had mixed success in pigs, with some authors reporting a relationship between measures of aggressiveness and those from non-social-confrontation tests, attributing this to differences in coping strategies (e.g. Hessing et al., 1993). Whilst the repeatability of these findings has been questioned by others (e.g. Forkman et al., 1995; Geverink et al., 2002; Janczak et al., 2003a).

To understand aggression in the context of personality, commercially indoor-reared pigs were studied at numerous key points throughout their lives to examine the prevalence of aggression and consistency within and between social confrontation situations. The effects of age and experience were also considered. Additional information was gathered to gain insight into the overall way in which these animals behaved in social and a non-social context. This information included non-aggressive behaviours, fighting success, injuries received, social status and salivary cortisol as measure of hypothalamic-pituitary-adrenocortical-axis activity to indicate relative stress responses. Maternal behaviour pre and post parturition was also studied, as a non-social-confrontation situation for comparison with aggressiveness, as it has important consequences for welfare and production.

The main findings of the four experimental chapters are summarised below, followed by a short discussion on the overall significance of these results and some questions raised by the results that may be interesting topics for future research.

6.1 Chapter summaries

6.1.1 Chapter 2, Do age and experience matter? Consistency of aggressive responses and the effect of repeated Resident Intruder Tests on pigs

Being aggressive in the resident intruder test (RIT) was the most common response. Nearly two thirds of RIT ended with an attack by the resident and being a consistent attacker was more common than being a consistent non-attacker. It was not clear whether aggressiveness was a more consistent trait or whether the definition of non-aggressive pigs was too stringent (few as opposed to no attacks might be a better definition). Individuals were consistent in speed and occurrence of attacking, but there were considerable differences between sexes suggesting that consistency occurred for different reasons; females were consistent attackers, whilst males were more likely to be non-attackers due to consistent mounting. Mounting was consistent from a very young age, prior to sexual maturity, and there appeared to a connection between mounting and mix-aggressiveness, although there was no link between mix aggression and RIT attack latency. Experience of the RIT seemed to improve consistency within and across tests, but did not affect the speed at which the pigs attacked, although again there was an effect of sex; experienced male pigs were faster to attack and less likely to mount, whereas female latency was unaffected. Early first experience pigs were more likely to attack, were faster to attack and were more consistent than late first experience pigs. This was confirmed by the finding that an early but distant previous experience had a greater effect than a more recent experience when older.

6.1.2 Chapter 3, Behavioural strategies during mixing at weaning in male and female pigs of differing aggressiveness; effects on fighting success, injuries received, dominance status and cortisol

The first 30 minutes after mixing was characterised by a period of high activity, with a smaller rise in aggression initiated by male pigs during the afternoon. Over time there was an increase in the duration, but not the frequency, of individual bouts of one-sided attacks, indicative of the establishment of a dominance hierarchy. As with Chapter 2 there were clear sex-differences, males being more aggressive, faster to perform aggression and more successful in fights than females. Mounting and play were also highly sex-linked; males were more likely to mount whilst females were more likely to play. Pigs were initially indifferent to the identity of lying partners, although this changed rapidly; suggesting that although at first, pigs were socially naïve, they learnt quickly to prefer littermates. Unfamiliar pigs received more aggression, attacks, short-non-aggressive-interactions (SNAI) and mounting during the first time interval (30 minutes post-mixing), although familiar pigs received more short-aggressive-interactions (SAI) and SNAI after this. The high levels of SAI between familiar littermates may have been a result of the frequently observed play-fighting sequences that can contain mild aggressive acts, or the formation of previously unestablished dominance ranks within litter. Cortisol analysis indicated that subordinate pigs found mixing more stressful. In addition to those pigs that received aggression and were unsuccessful in fights, there was another group, which avoided other pigs and subsequently avoided conflict. These pigs had lower increases in cortisol post-mixing and lower cortisol several days after. The most aggressive pigs had a greater number of skin lesions.

There would have been considerable opportunity for pigs to re-adjust their status between the aggression seen during the post-weaning mix period and the pre-slaughter dominance test; nonetheless rank was to a certain extent predictable, with a weak relationship between aggression and success in the mix at weaning and subsequent dominance. In addition to being more aggressive, male pigs were heavier and more dominant than females. It seems likely that fighting ability and subsequent dominance result from a combination of various factors, of which weight and aggressiveness are major contributors. Aggression during mixing was not predictive of RIT latency to attack; in fact high-mix aggression pigs were slower to attack in RIT3 than low-mix aggression pigs. This was influenced by sex and was in part confounded by the effect of mounting by males (see Chapter 2). The link between mix-aggression and mounting discussed in Chapter 2 was confirmed.

The two most dominant behaviour dimensions of the weaning mix described: overt aggressiveness, including fighting and attacking ('Attacking'); and playful-shorter-interactions, which included both aggressive and non-aggressive behaviours, such as, short aggressive interactions (SAI) and playing ('Play'). Using Principle Components Analysis (PCA) the pattern of behaviours alluded to different strategies employed by the pigs, the extremes of which were high playfulness (low aggression) and high aggressiveness (low play). As would be expected, the aggressive individuals were involved in more fights and won more fights, but suffered more skin lesions. Pigs that engaged in high 'playing' did suffer injuries (lesions), although these were not as severe as those engaged in high levels of overt aggression. Playful pigs were generally the least successful in fights, however this did not translate to lower pre-slaughter dominance rank. In fact gaining higher dominance was associated with pigs displaying both play and aggression, as low ranking pigs did not exhibit high levels of either. Even though more males were classified as aggressive and more females as playful, the differences in strategy could not be explained by the differences in weight observed between sexes.

6.1.3 Chapter 4, Aggressiveness in female pigs, comparisons between behavioural strategies of female pigs mixed at weaning and as gilts

Analysis of the gilt data (a subset of females from previous Chapters) showed similar levels of consistency within RIT4 and between all of the RIT, as was found for all the female pigs in Chapter 2. Again, attacking was by far the most frequent outcome across all tests (75 to 81 %). The only notable change in latency to attack was after RIT 1a, probably as a result of the novelty of the first testing experience. Even with a gap of 90 days between RIT 3 and 4 (including the onset of puberty) the speed of attacking did not differ significantly. RIT aggressiveness was not predictive of subsequent aggressiveness at mixing.

There were some similarities in the behaviour of pigs mixed at weaning (Chapter 3) and then again as gilts; behaviour frequencies peaked during the first 30-minute interval and the most frequent behaviours were SNAI and SAI. There was also a second rise in aggression during the afternoon in the all-female mixes, even though it had been the male pigs that were responsible for the second peak in aggression during the weaning mix. Fighting ceased sooner in the gilt mix compared to at weaning. The gilts had received several opportunities to gain social experience from previous testing and evidence from the literature suggests that

this experience may have resulted in the accelerated resolution of disputes (compared to at weaning). Alternatively, aggression may have ceased earlier because group sizes were smaller. Adjustments for group size found there was no difference in fighting, however pigs were faster to perform an aggressive behaviour as gilts and spent more time attacking and pushing. There was more aggression overall in the gilt mix, including attacking body, which is a particularly severe and injurious form of unretaliated aggression. Injuries were more frequent in the gilt mix and more lesions were found to the front (head, ears, and shoulders), indicative of mutual fighting behaviour. This suggests that although aggression ceased sooner during the gilt-mix, it may have been more intense.

As well as differences in the frequencies of particular behaviours, the overall behavioural structure changed between mixes. The most dominant PCA factor dimension in both mixes described aggression. The second dimension at weaning represented short interactions and in the gilt mix was described by avoiding. SAI, performed during play in weaned pigs, were associated with serious aggression as gilts. The playful pigs at weaning adopted a strategy of low aggression but also low avoiding as gilts. Aggression was not directly linked between mixes, which may have been due to the differences in the behavioural and physical structure of the mixes (e.g. no males present). Within the gilt mix there were clear effects of dominance, for example, pigs that went on to be classed as high-ranking individuals were more aggressive, had fewer lesions and were the least preferred lying partners. Cortisol increased 30 minutes after mixing, but returned to baseline levels by 24 hours post mixing. Pigs that received more attacks and lost more fights had higher pre-mix cortisol but a lower increase 24 post-mixing and lower cortisol seven days later, whereas more dominant and aggressive pigs showed the opposite pattern.

6.1.4 Chapter 5, Does having an aggressive personality effect maternal ability?

The pre-farrowing period was characterised by frequent posture changes and nest-building, with a gradual progression towards immobility before parturition. The exception to this was pawing the ground, which increased as farrowing approached. There are several possible explanations for this; it may have been a throwback to naturally occurring nesting-behaviour, the result of continued motivation to nest from a lack of nesting material in the crate, or an indicator of contraction pain. As reported in other studies gilts tended to sniff or look at the first piglets born and then lie in lateral recumbency for several hours, possibly as the result of

a number of physiological processes, such as opioid-mediated passivity. Approximately 28 % of gilts reacted to piglets with aggression of varying severity; two gilts attacked piglets and another performed behaviours that could have directly caused piglet deaths (9.4 %), whereas six others performed a small number of aggressive behaviours (18.8 %), but generally posed no real danger to their piglets. The six mildly aggressive gilts were not considered to be savagers, but were more 'reactive' during farrowing. There was no clear link between pre-farrowing behaviours and subsequent maternal aggression, although as would be expected, maternally aggressive sows were more active after the onset of farrowing and more responsive to piglets.

Pre-farrowing and post-farrowing behaviours were linked to some of the measures of aggressiveness from the gilt-mix and RIT; frequent posture changes pre-farrowing were made by more aggressive and successful pigs, whereas nesting was more frequently performed by less aggressive gilts. Gilts that were aggressive and successful in the mix showed higher levels of maternal aggression and there appeared to be a connection between the experience of being mixed as gilts and maternal aggression (33.3 % mixed were MA, 18.2 % unmixed), although this wasn't significant. Cortisol levels were greatest in gilts three to five days pre-farrowing (before being moved into farrowing crates), followed by 12-24 hours after farrowing. Reasons for this pre-farrowing rise in cortisol could include responses to environmental stressors, such as lack of feed or space to roam from the social group, the effects of which may have been exaggerated due to imminent farrowing. There was an indirect link between lower cortisol 37 days before farrowing, more frequent rooting behaviour pre-farrowing and being non-maternally-aggressive. None of the gilts were considered as excessively aggressive towards the stock-person in the two-week period following farrowing. There was no link with maternal aggression and stock-person-directed aggression suggesting that the two different types of aggression may be provoked by differing emotions, such as anxiety or fear.

6.2 Points for discussion or future study/analyses

6.2.1 Consistency, learning and experience

This study demonstrated consistency in aggressive behaviour, although consistency was more apparent between identical testing situations (such as the RIT) and less so in tests where the dynamic had changed in some way, such as the removal of the males in the dominance tests and mixing as gilts. Being aggressive was more common than being non-aggressive in the RIT. It may however, have been unreasonable to expect even the most subordinate of pigs to never exhibit aggression, especially as the RIT gave them the advantage of size and home territory. Aggression can also be displayed as a form of defence, rather than offence and it has been suggested that "*a minimum amount of aggression in the animals is protected by the genotype*" (van Oortmerssen & Bakker, 1981), as it would not be sensible from an adaptive perspective never to be aggressive. Some aspects of human personality are stable, whereas others are more 'dynamic' in nature (Roberts & DelVecchio, 2000); thus it is possible that whilst being highly aggressive is a stable trait of individual pigs, the propensity to exhibit aggression in the non- or moderately-aggressive pigs is more changeable and situation dependent. In addition to the influence of trait-aggression, it is possible that the RIT is rewarding to attacking pigs, priming them to continue being aggressive in the future. The fights were separated without serious injuries being inflicted and resulted in the intruding pig being removed and so the attacking resident may have perceived this as a 'win'. Winner (and loser) effects were not investigated here, but they have been widely reported (Chase et al., 1994; Hsu & Wolf, 1999; Oyegbile & Marler, 2005). Interestingly, a link has been made between winning and higher testosterone levels in mice (Oyegbile & Marler, 2005). High levels of testosterone may be associated with both aggressiveness and mounting (Cronin et al., 2003) and in this study, although testosterone was not measured, aggressiveness and mounting behaviour were connected. This relationship between winning, aggressiveness and hormone levels may be an interesting area for future research.

One of the main and unexpected findings in this study was the disparity between male and female pigs in behaviours including playing, aggression and mounting and also that these differences were evident from a young age (pre-puberty). Such differences are rarely reported in the literature. Spinka (2001) suggested that male young of sexually dimorphic species should play more than females, as play prepares them for more serious conflict. The

behavioural ecology of free-ranging/wild pigs differs between males and females once they reach maturity. Females naturally live in socially structured groups, whilst males live alone, or in small groups of males up to about three years of age (Gonyou, 2001). Boars joining the female social groups occupy the highest rank (Gonyou, 2001) and may have to fend off challenges from rival boars (Watson, 2004), but generally aggression in mature pigs is not common. Sex differences between juvenile free-ranging pigs of a comparable age to this study have not been reported and in contrast to the suggestion of Spinka et al. (2001) it was the female pigs, not the males that played more. It has been suggested that in humans the extent of the influence of nature (genetics), relative to nurture, on any given trait may differ between sexes (van Beijsterveldt et al., 2003); perhaps this also occurs in pigs. Breeding-line (dam-line) semen from just two boars, rather than pooled meat-pig (terminal-line) semen, was used. It may be that the male offspring of these breeding boars were generally more prone to aggression, for example, as a result of higher testosterone compared to meat-pigs, whereas the female pigs were unaffected. Without evidence this is purely supposition, however it would be possible to test this hypothesis experimentally and links between the aggressiveness of sons with their fathers have been demonstrated in mice (Van Zegeren, 1980).

The pigs learnt very quickly about the testing procedures. This was evident in the repeated tests (RIT and the dominance tests on the gilts), where they were observed to get extremely 'excited' during the setting up of the equipment. Unfortunately this was unavoidable as these tests were performed in the home pens. Any pre-test anticipatory excitement did not however, appear to decrease latency to attack in the RIT and it also seems unlikely that this behaviour altered the outcome of the dominance tests. Nonetheless the results presented confirm that the effect of learning and experience should be not be discounted when using repeated behavioural tests. As Chapter 2 demonstrated, this is a particularly important consideration when testing starts at a young age.

Many of the relationships between variables, such as attack latency (Chapter 2), were considerably weaker or were lost altogether when treatments or sexes were analysed separately. One cause of this was the reduction in sample size, as generally larger sample sizes improve the power of analyses, particularly in tests such as Spearman's Rank Order Correlation where significance is directly linked to sample size. The sample sizes were lower than expected as mortality was higher than expected. Pre- and post-weaning mortality rates were 15.6 and 8 % respectively, with an average loss of two live-born piglets per litter. The

pre-weaning mortality rate was slightly higher than mean mortality in commercial conditions, but was within the normal range (mean 12 %, range 5-25 %, Carr, 2006). It may be that the additional interference from experimental procedures and the extra health-checks that the study piglets had to undergo, compared to the farm pigs, caused an increase in stress-related illness or disease transmission between pens. Another contributing factor was that all of the mothers were gilts, and as savaging/maternal aggression is more common in gilts (Harris et al., 2003) then piglet losses might be greater than expected compared to experienced sows. The gilts caused the death of 6.8 % of piglets, through crushing, savaging or from injures that were either fatal or led to piglets being put down (e.g. being stood on). The second highest cause of pre-weaning mortality (5.6 %) was from low-viability (runt) piglets and starvation. The remaining pre-weaning deaths (3 %) resulted from lameness, scouring, birth deformities and undetermined causes. The majority of post-weaning deaths resulted from Post-weaning Multi-systemic Wasting Syndrome (PMWS). This disease attacks the immune system of infected pigs and the majority of these pigs will die, or have to be destroyed, as there is no effective treatment (although some of the secondary infections can be treated). Little is known about the causes and spread of PMWS, or why only certain pigs within a group succumb to the disease. Sickness was usually observed shortly after groups were moved into the grower accommodation (between two and four weeks post-mixing). Stress has been proposed as one of the contributing factors (Carr, 2006; Meat and Livestock commission communication April 2002/02183 141/10M). As the experimental animals received additional social confrontation tests (RIT) it would therefore have been expected that they would have had a higher mortality rate than the farm pigs. However this was not the case and there was no link between the number of test procedures received and the development of PMWS. In fact, sickness was often evident before the first RIT and therefore precluded these animals from being tested at all. During the peak outbreak of PMWS mortality-rates were between 6 and 7 %, which is relatively low, as typically between 5 and 20 % of an afflicted herd can be infected (Carr, 2006). Generally the pigs at EHPU are not overly stressed; they are housed at, or below, commercial stocking densities, have straw bedding and growing meat-pigs (i.e. not breeding pigs) are generally mixed into social groups only once on farm (they are also are usually mixed during transport to slaughter). There was a peak at the start of the outbreak, which also corresponded with the change to cooler weather in the autumn/winter of 2003 and so environmental stress may have been a contributing factor. Mortality rates settled 6-12 months after the initial outbreak. In the light of evidence for personality traits in pigs and the corresponding differences in stress reactivity, vulnerability to immune challenges, such as PMWS, should be studied in

great detail, as links between behaviour, stress susceptibility and poor immune function could have an immense impact on welfare, productivity and husbandry practices.

6.2.2 Behaviour strategies during mixing

There were several aspects of behaviour at mixing that would be interesting areas for future investigation. Dominance is usually related to weight, although this is often not a linear relationship. Body dimension measurements were taken as a refinement of weight as an indicator of resource holding potential. For example, pigs with greater muscle mass in particular areas (such as the shoulders) might have (or be perceived to have by their opponents) better fighting ability than pigs of the same weight and similarly, heavier pigs may not be more successful if they are in poorer condition. The relationship between body dimensions as an indicator of RHP and measures of aggressiveness or success was not explored fully here and it may be that either more sophisticated analyses, or measurement techniques, such as visual imaging (e.g. Whittemore & Schofield, 2000), are needed to do this.

Aggression and subsequent dominance appeared to be linked; however, there was little evidence for a link between RIT aggression at any time-point and aggression displayed during the mixes. The RIT did seem to produce a consistent measure of RIT aggressiveness, but the usefulness of this is limited if it does not relate to mixing aggressiveness, as the latter situation, and subsequent detrimental effects, were the motivation behind this study. The mix situation in pigs was perhaps too complex to be fully predicted by a simple RIT test. The RIT was initially developed in rodents and from a functional perspective, the RIT may be a better simulation of social conflict situations in rodents. As pigs generally live in social groups (older males can be solitary), a dispute over territory between a single resident versus a single intruder, may simply be irrelevant in pigs. It could be argued that the RIT should still indicate which animals are inherently more aggressive; and strategies during mixing are likely to be influenced by aggressive personality. However, during mixing, pigs are confronted by several unfamiliar conspecifics, as well as having other familiar conspecifics present, and both of these factors may stimulate or suppress aggression to varying degrees between individuals. Mixing situations where the experimental pigs had an advantage over opponents, such as greater numbers or size, might have found a clearer link between RIT and mixing aggressiveness. Alternatively, more sophisticated analyses may be able to determine whether RIT aggression explains more of the aggressiveness seen in the mix. For example,

in addition to the overall frequency and duration measures taken here and in other studies, the sequence of aggression is likely to be an important factor to consider. D'Eath (2002) found that RIT aggressiveness correlated with some measures of mixing aggression on the day following mixing, but not on the day of mixing itself. Meese & Ewbank (1973) referred to differences in the timing of aggression according to subsequent dominance. Thus, studying only the initial period following mixing may not provide sufficient information about relative aggressiveness; looking at the sequence of fighting over a longer period may help to unravel the relationship between relative aggressiveness and social status. Whilst the analyses here were divided into intervals, it may be worthwhile extending this time-interval-based analysis beyond the first day post-mixing.

Another use for detailed sequence-analysis would be to examine whether post-conflict reconciliation behaviour occurs in pigs. Friendly post-conflict behaviours may be particularly important in species that live in stable social groups as a means to confirm that a pairing has been settled, reducing uncertainty, anxiety and further conflict between opponents (Aureli et al., 2002). Studies of post-conflict reconciliation and social bonding have mainly focused on primate species (e.g. Roeder et al., 2002; Whitham & Maestripieri, 2003; Wittig & Boesch, 2005), although affiliative behaviours (greeting and non-aggressive approach) have been reported in Hyenas (Wahaj et al., 2001) and also in goats (muzzle-muzzle/body; Schino, 1998). Observations during mixing indicated that SNAI seemed to occur before sitting down, with those pigs that directed SNAI towards a potential lying partner being less likely to receive an aggressive reaction after sitting. To substantiate whether this was an affiliative post-conflict behaviour, an analysis of the sequence of events would be required as well as a more detailed examination the behaviours classed as SNAI (e.g. sniff, chew, nose). Greeting behaviours in primates often involve high-risk behaviours as a test of social bonds (Whitham & Maestripieri, 2003), with the social partners benefiting from such close affiliations (Maestripieri, 2000). It has been suggested that pigs do form preferential associations (Durrell et al., 2004) and SNAI directed to the head of another pig could be considered a risky behaviour, as it can result in aggression by either the actor or recipient. SNAI was a frequent behaviour in both mixes. In the weaning mix SNAI was associated with the high-play-low-aggression strategy pigs, which then went on to attain similar status as the high-aggression strategy pigs. It may therefore be worthwhile investigating the nature of these relationships and specifically whether affiliative alliances are an alternative approach to aggression in attaining higher social status. Without further investigation, the exact role of this behaviour is equivocal; is it simply a method of

identifying familiar/unfamiliar pigs, the preliminary stages of exploring relative RHP (Jensen & Yngvesson, 1998), or a reconciliatory or bonding behaviour?

The gilts had all been exposed to a similar number of social conflict situations prior to mixing (32 gilts from treatment 123, four from treatments 13 and 23). Thus it was only possible to theorise that the differences in the structure of aggression in recently mixed gilts, compared to when weaned, were due in part to increased social skills. This supposition was based on the findings of other authors and evidence in this thesis for an effect of repeated testing improving consistency of RIT aggression. It would be interesting to compare mixed-gilts according to differing experience, with a control group without any prior experience of the RIT (or mixing). Using this approach it may be possible to clarify whether previous social conflict experience alters aggressiveness and the speed of hierarchy formation (and also how much experience is required), or if changes are simply a consequence of age/maturity and the social skills developed naturally in stable groups. In addition, experience of social conflict may have had an effect on gilt-piglet interactions, as proportionally more mixed pigs were maternally aggressive, although a larger sample size would be required to establish if this trend was a chance effect. A study based on maternal behaviour in relation to mixing experience would be relevant both in terms of welfare and production, as breeding sows generally experience mixing into new social groups frequently. Detrimental effects of social mixing during pregnancy have demonstrated (e.g. Jarvis et al, 2006); but in this study, the gilts were mixed into new social groups as pubescent animals, with potential differences in maternal behaviour seen several months later. Evidence for an increased risk of piglet-directed aggression in mixed animals might encourage changes to husbandry practices, which minimise the number of times (or timing) that breeding animals (or young pigs destined to become breeding sows) are mixed into new social groups.

6.2.3 Coping and aggressiveness as part of personality

Ideally more measures of social and non-social challenges would have been taken to get a better understanding of aggressiveness within the wider context of personality. To examine true lifetime aggression it would also have been useful to look at pre-weaning aggression. Another opportunity to study aggression would have been pre-weaning as piglets initially exhibit considerable competition over teats. It has been proposed that aggression at the udder is more akin to territorial aggression (Drummond, 2006), and so this could potentially have

provided information on aggressiveness with a different functional motivation to that seen in the tests of social confrontation with strangers.

A link between gilt-mix-aggression and maternal aggression was demonstrated, which was an important finding with consequences for welfare and production. There appears to be very little in the literature about how trait-aggressiveness may affect maternal ability. More attention has been focused on the neural or physiological process behind infant abuse (e.g. Johns et al., 1994; Maestripieri, 1999) and how maternal behaviour can alter the aggressive behaviour of offspring (e.g. Stocker et al., 1989; Meaney, 2001; Lyons-Ruth et al; 1993). The relationship between cause and effect should be examined more closely. It could be that maternal abuse leads to more aggressive offspring, which go on to be abusive parents themselves, as a result of the experience of being abused (i.e. without any genetic influence). For example, in mice, a link has been made between parental behaviour, with subsequent offspring aggressiveness in a RIT and a neutral arena (Bester-Meredith & Marler, 2003). Alternatively, there may be a heritable link between aggressive and parental traits through personality, if for example, they are mediated by similar physiological mechanisms (e.g. neurochemicals, or hormones such as vasopressin and progesterone) (Marler at al., 2005).

It would have been interesting to further investigate the relationship between maternal behaviour and prior mixing behavioural strategies that emerged from the wean mix (i.e. playful versus aggressive). Unfortunately there were too few gilts in the category 'aggressive' to perform any meaningful analyses (N = 4), nonetheless, examination of the means indicated that there may have been differences in behaviours pre and post farrowing and potentially differences in the likelihood of maternal aggression. These findings warrant further investigation: not only as they demonstrate that aggression should not be studied in isolation, but should be considered in a wider context with other personality traits; but also because using measures of reactivity in a social context may provide indicators for animals that will better cope with the farrowing crate environment. This is not to say that aggressive individuals should not be used for breeding, as with further research it may become evident that these pigs cope better with other farrowing environments, in the same way that proactive and reactive types of rodents may fair better in different conditions. This would require a much larger-scale study than here, using 32 gilts was insufficient, predominantly as only a small number of gilts were classed as 'aggressive' during mixing at weaning.

Little evidence was found here for the 'coping strategies' seen in rodents, however, a limited number of non-aggression measures were taken (e.g. maternal reactivity and cortisol) and a greater spectrum of measures would have been beneficial. Coping strategies cannot entirely be dismissed, but it seems likely that there are more than just two types of individual, with proactive and reactive being the most divergent personality types along one particular axis of measurement. If aggressiveness forms one part of a multi-dimensional personality profile in pigs then simply counting behaviours that we recognise as aggression may not be sufficient to distinguish 'aggressive' individuals. Aggressive behaviours may change in their functional meaning, particular behaviours that characterise aggressiveness at one life-stage may change with age or maturity e.g. SAI. Similarly, other seemingly unrelated behaviours, such as SNAI or maternal behaviour may in fact be linked to aggressiveness. Other influences on the motivation to perform an aggressive behaviour, such as fear or stress (Jensen, 1994), will alter those aspects of personality that are expressed at any one point. In humans there are reported to be different types of aggressiveness, hostile (reactive and impulsive) versus instrumental aggression (proactive, premeditated and controlled) (Ramirez, 2006). Studies of this nature might be difficult to achieve in pigs, but may be worth future investigation to help understand some of the contradictions in the study of aggressiveness, such as why some subordinate pigs continue in being aggressive without any obvious benefit. Although studies demonstrating personality or behaviour strategies have not been universally successful, considering the growing evidence for personality types across many species (Gosling & John, 1999), emphasis should continue to be placed on examining a wide range of variables. Where evidence is found for links between traits they should not be studied in isolation (Sih et al., 2004). It is also important to not prematurely amalgamate behaviours that are assumed to have the same meaning, as demonstrated in this study (e.g. SAI differed from attacking at weaning). Finally, it is worth remembering that in humans at least, personality is not a totally rigid entity, but is changeable with time (Roberts & DelVecchio, 2000), development and experience. Personality development is also likely to differ between sexes; in addition, the relative influence of environmental and genetic factors may differ between sexes. In a study of human aggression development, the influence of genetic and environmental factors differed between boys and girls (van Beijsterveldt et al., 2003), with environment factors more influential in girls and genetics more pertinent in boys. This could have particular relevance to pigs in light of the differences found between the sexes here. The semen used to produce the pigs was from two dam-line boars, selected on the basis that they would produce higher quality daughters for breeding, but also based upon reproductive measures of the sires (e.g. semen quantity and libido). The differences in aggression between the sexes and the

early appearance of sexual-type behaviour (mounting) in the males may have resulted as a by-product of the breeding selection process in the sire generation, with the influence of genetic factors potentially greater in the male progeny. Environmental influences may have been greater in the females, for example, experience of the RIT did appear to result in shorter attack latencies in last test (Chapter 2) in the female pigs. Further investigation of any 'boar' effects would be required to substantiate this theory.

6.3 Summary

Pigs were consistent in responding to the RIT. Responses were altered by age and experience: early first experience increased the likelihood and speed of attacking; experience increased consistency. Males took longer to attack as they got older, whereas latency did not vary in the females. Aggression was a consistent trait of individual pigs, although aggressiveness was not a unilateral trait as there was little evidence for RIT being predictive of mix-aggression. RIT does not therefore appear to be a gold-standard test of aggression, particularly in male pigs. Pigs adopted clear behavioural strategies during social mixing and mixing behaviour changed with age/maturity. Finally, aggressiveness during social mixes was linked to maternal behaviour, with aggressive pigs being more likely to be maternally aggressive towards their piglets. This last finding in particular suggests that a wider view needs to be taken of aggression within personality, rather than as a trait in isolation from other traits.

Aggressiveness and personality are not a fixed entities and a greater understanding is needed of how they develop with age, sex and environmental influences. Experience and age of experience appear to be particularly potent influences, which may also be sex-dependent. The differences in behavioural strategies seen during mixing, and the fact that some individuals managed to attain rank without severe fighting, or receiving excessive numbers of lesions, indicate that it is important not to focus on just the most aggressive individuals. It may be that aggressive pigs are more reactive in a number of situations; reactivity to piglets during farrowing in crates being one of these situations. There could be some benefit to breeding-out aggressiveness. However, as aggression appears to be a fundamental behaviour, even in low-aggressiveness pigs, it seems unlikely that it can be eliminated, particularly as it cannot be treated in isolation from other aspects of personality. The future of aggression studies should continue to increase our understanding of how aggressiveness relates to other traits of personality, particularly in the context of other factors relevant to welfare, such as maternal ability and immunological competence.

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"A righteous man regardeth the life of his beast: but the tender mercies of the wicked are cruel"

(The Book of Proverbs, 12:10)